Microbial bioreaction process

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(54) **MICROBIAL BIOREACTION PROCESS**

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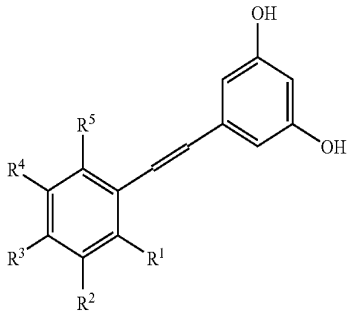
(57) **ABSTRACT**

A cis- or trans-stilbenoid of the general formula (1): in which each of R^1, R^2, R^3, R^4 and R^5 is hydrogen or hydroxy, or a glycosylated or oligomeric form thereof, is produced by cultivating a micro-organism producing said stilbenoid, in a multi-phase system comprising at least an aqueous first phase containing said micro-organism and a second phase immiscible with said aqueous phase in which (e.g. as which) said stilbenoid accumulates. The second phase may be said stilbenoid or a free or encapsulated solvent compatible with the growth of the micro-organism, for instance an ester.

(1)



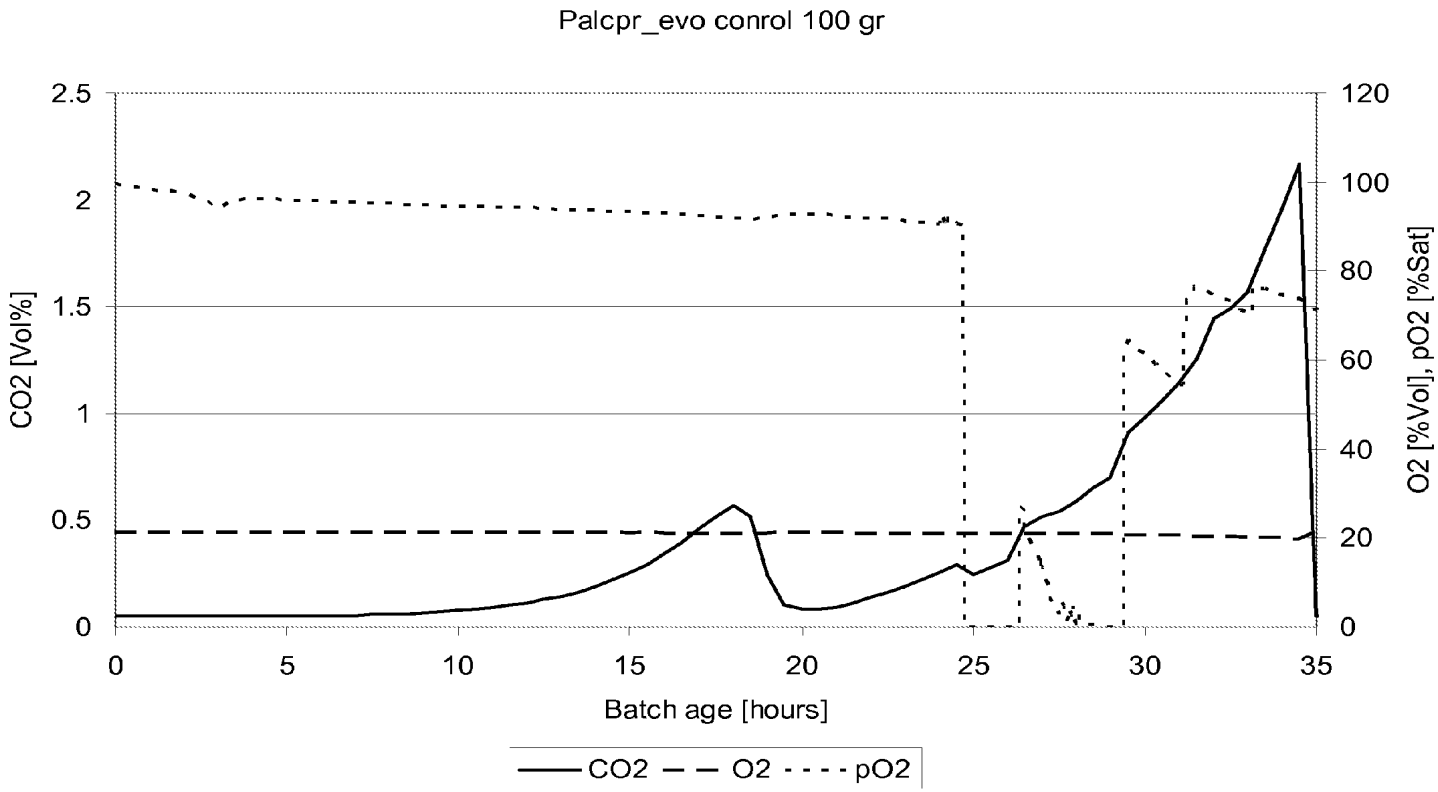

Palcpr_evo conrol 100 gr
CO2 [Vol%]
O2 [%Vol], pO2 [%Sat]
Batch age [hours]
CO2
O2
pO2
0
0.5
1
1.5
2
2.5
0
5
10
15
20
25
30
35
0
20
40
60
80
100
120

Figure 1A


Palcpr_evo solvent 100 gr
CO2 [Vol%]
O2 [%Vol], pO2 [%Sat]
Batch age [hours]
CO2
O2
pO2
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
120
100
80
60
40
20
0
0
5
10
15
20
25
30
35
40
45
50

Figure 1B

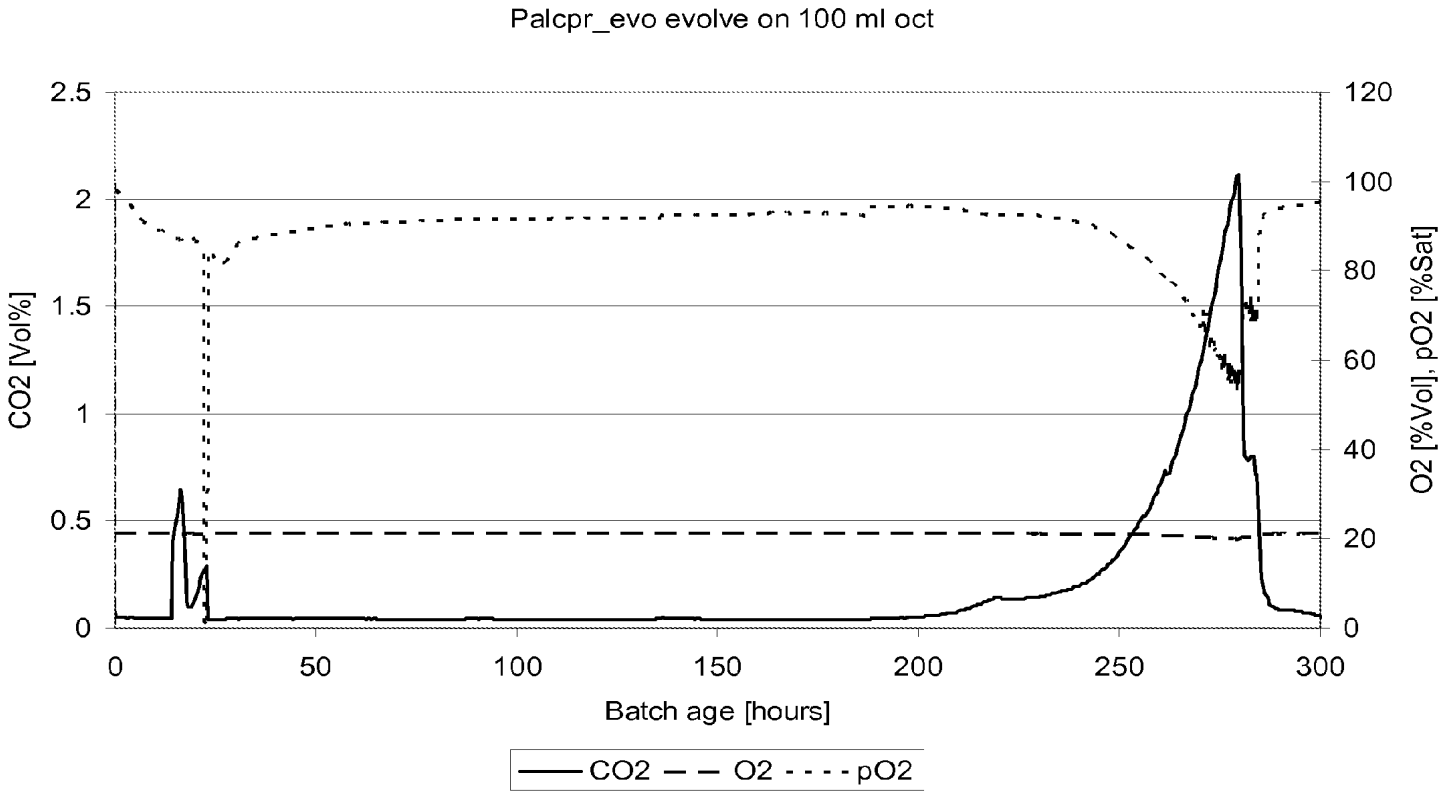

Palcpr_evo evolve on 100 ml oct
CO2 [Vol%]
O2 [%Vol], pO2 [%Sat]
Batch age [hours]
0
0.5
1
1.5
2
2.5
0
20
40
60
80
100
120
0
50
100
150
200
250
300
CO2
O2
pO2

Figure 2

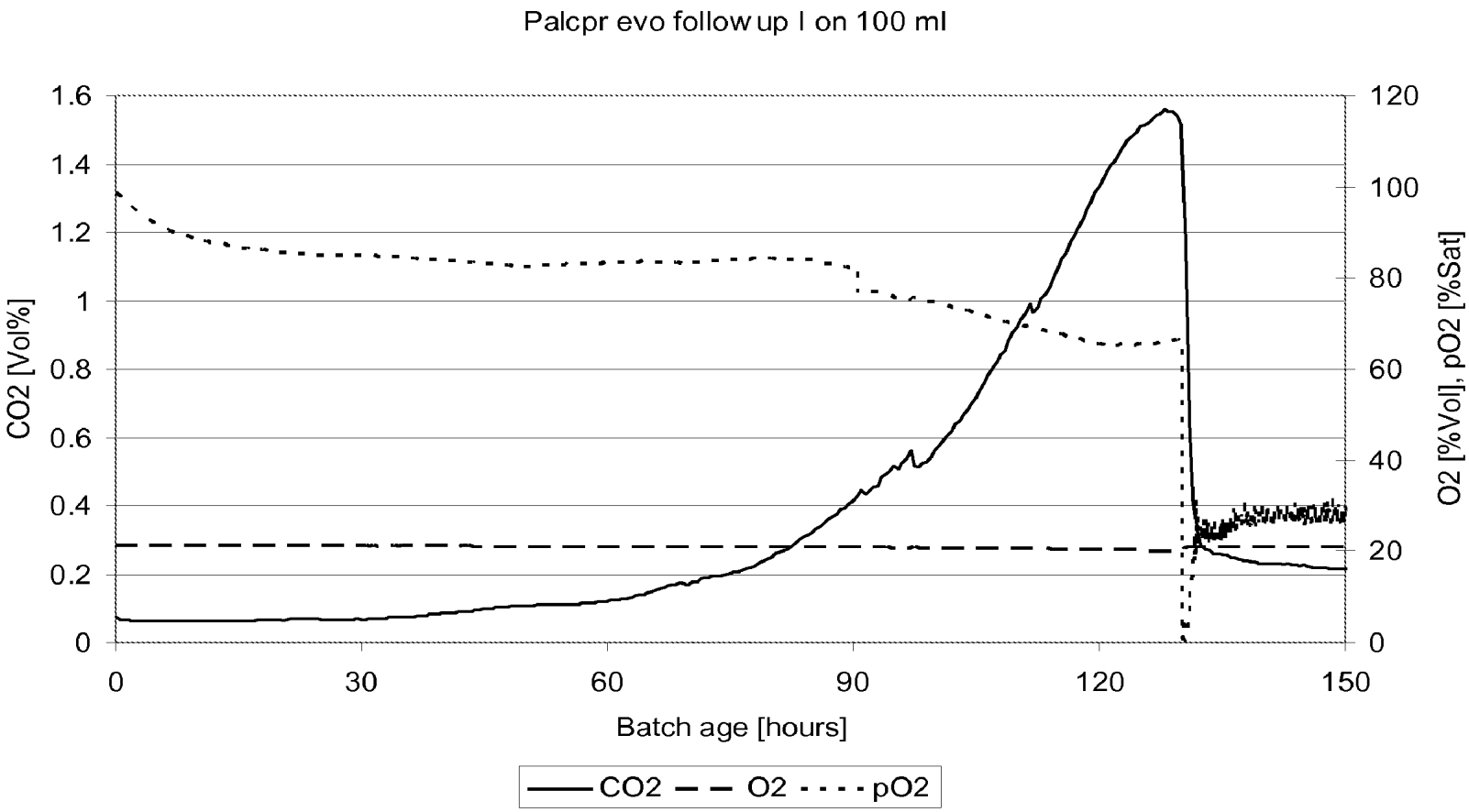

Palcpr evo follow up I on 100 ml
CO2 [Vol%]
O2 [%Vol], pO2 [%Sat]
Batch age [hours]
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
0
20
40
60
80
100
120
0
30
60
90
120
150
CO2
O2
pO2

Figure 3

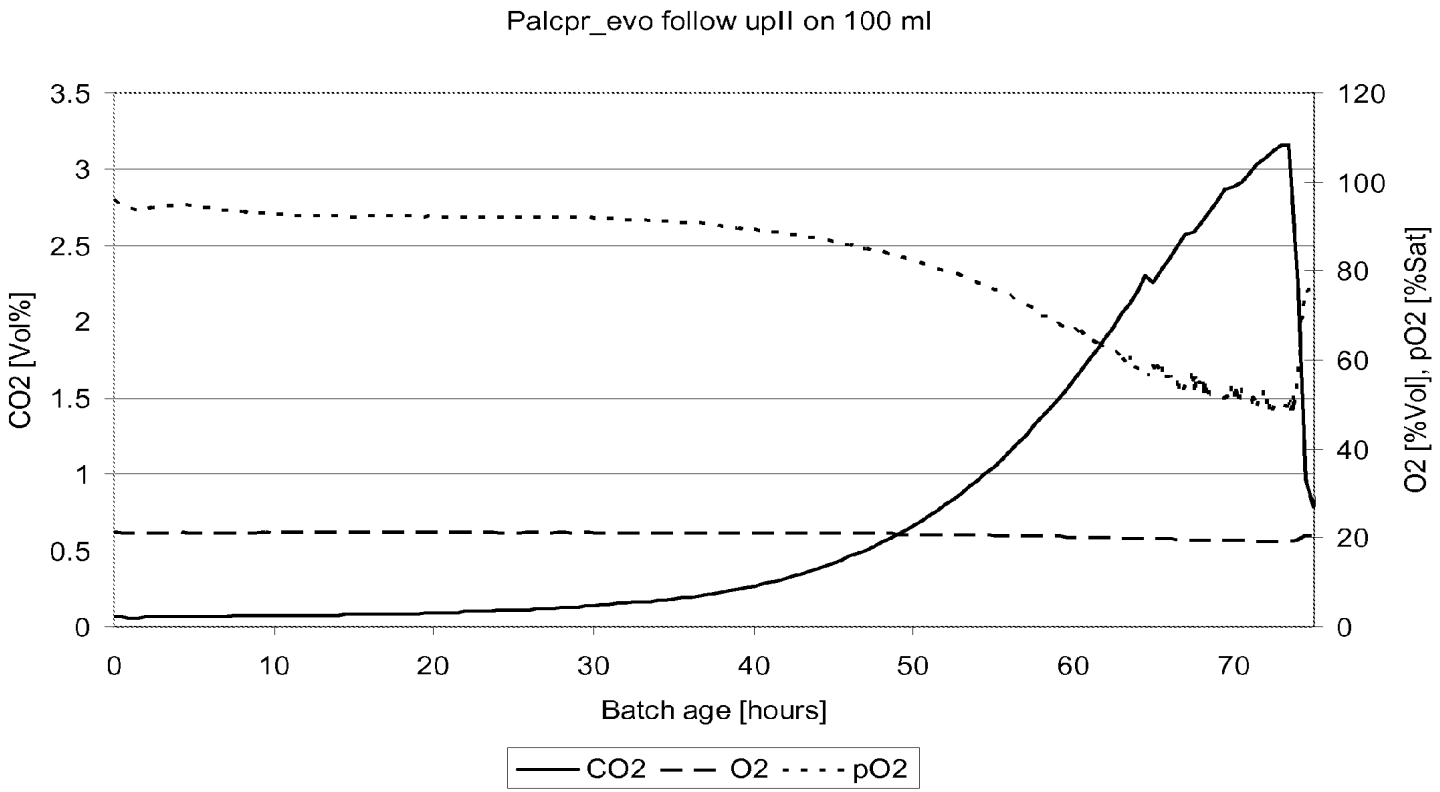

Palcpr_evo follow upII on 100 ml
CO2 [Vol%]
O2 [%Vol], pO2 [%Sat]
Batch age [hours]
CO2
O2
pO2
0
0.5
1
1.5
2
2.5
3
3.5
0
20
40
60
80
100
120
0
10
20
30
40
50
60
70

Figure 4

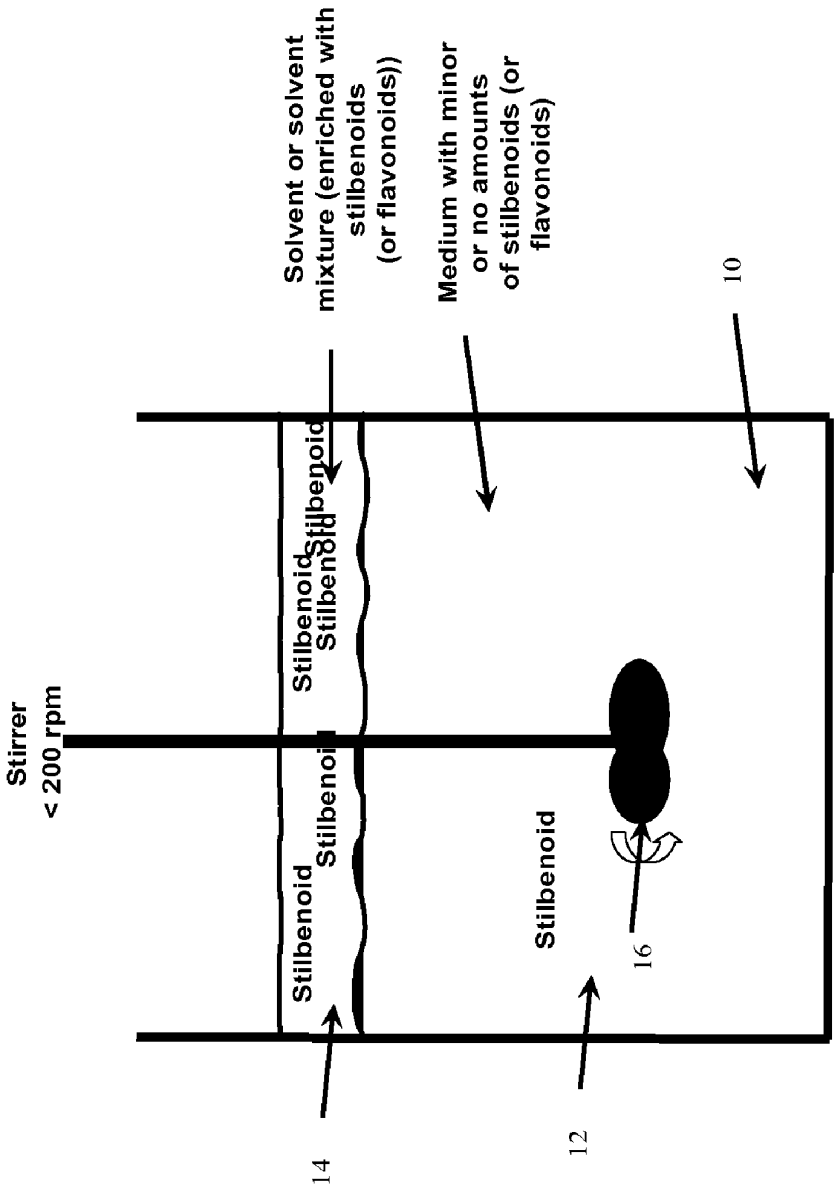

Solvent or solvent mixture (enriched with stilbenoids (or flavonoids))
Medium with minor or no amounts of stilbenoids (or flavonoids)
10
Stirrer
< 200 rpm
Stilbenoid
Stilbenoid
Stilbenoid
Stilbenoid
Stilbenoid
16
14
12

Figure 5A

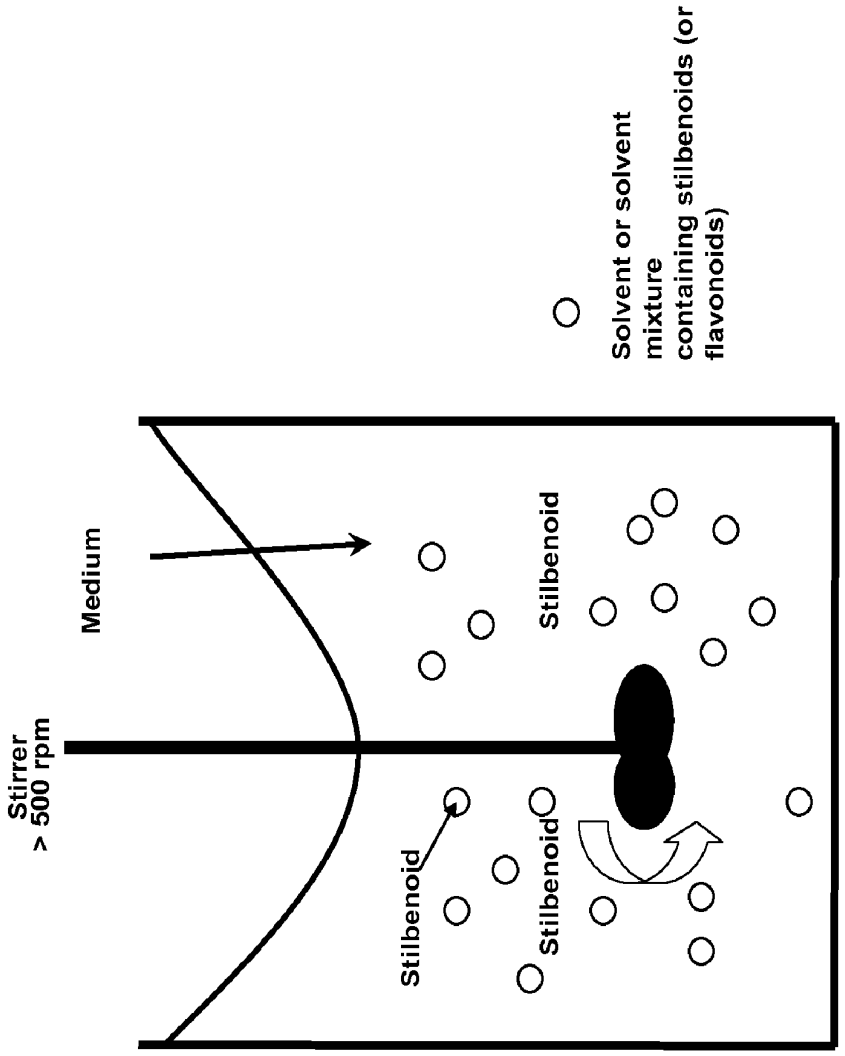

Solvent or solvent mixture containing stilbenoids (or flavonoids)
Medium
Stilbenoid
Stirrer
> 500 rpm
Stilbenoid
Stilbenoid

Figure 5B

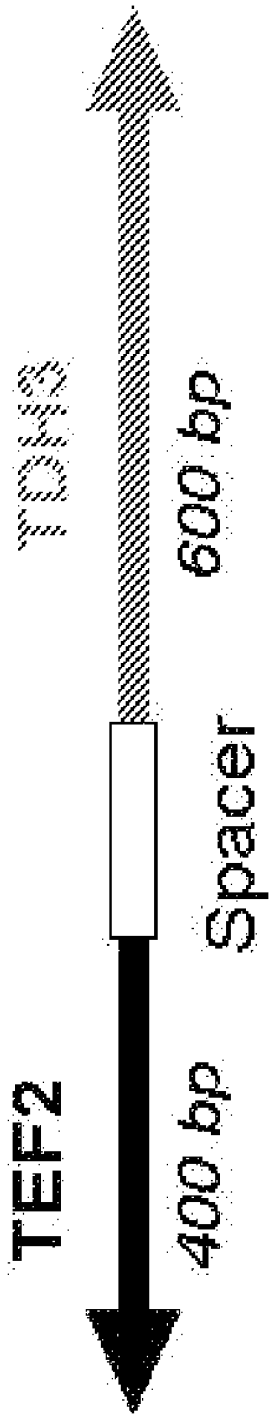


Figure 6:

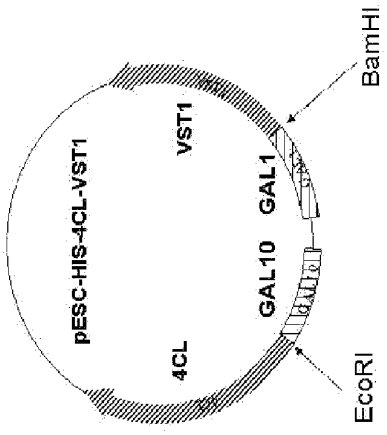


Figure 7

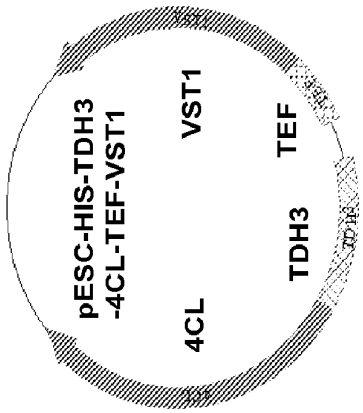


Figure 8

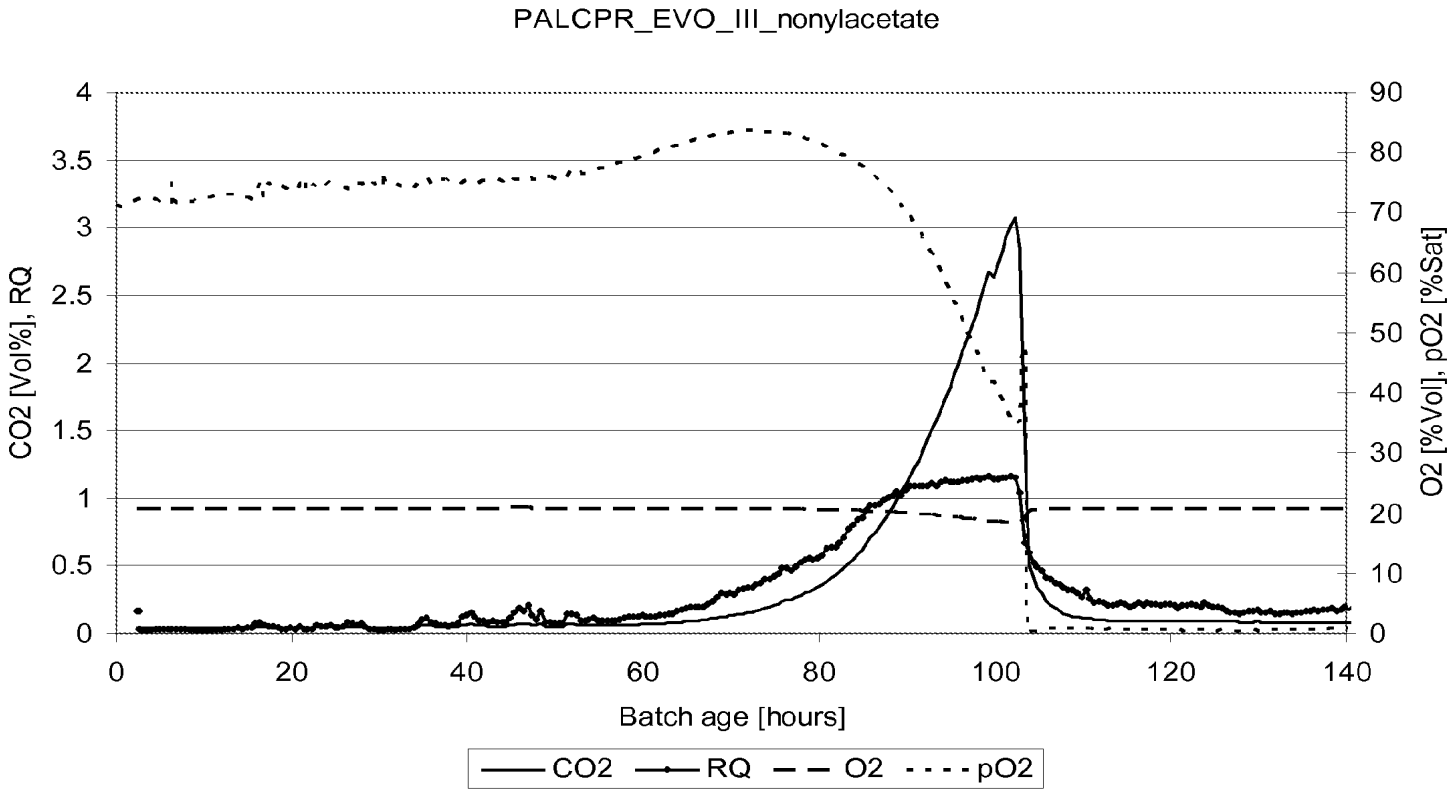

PALCPR_EVO_III_nonylacetate
CO2 [Vol%], RQ
O2 [%Vol], pO2 [%Sat]
Batch age [hours]
0
0.5
1
1.5
2
2.5
3
3.5
4
0
10
20
30
40
50
60
70
80
90
0
20
40
60
80
100
120
140
CO2
RQ
O2
pO2

Figure 9

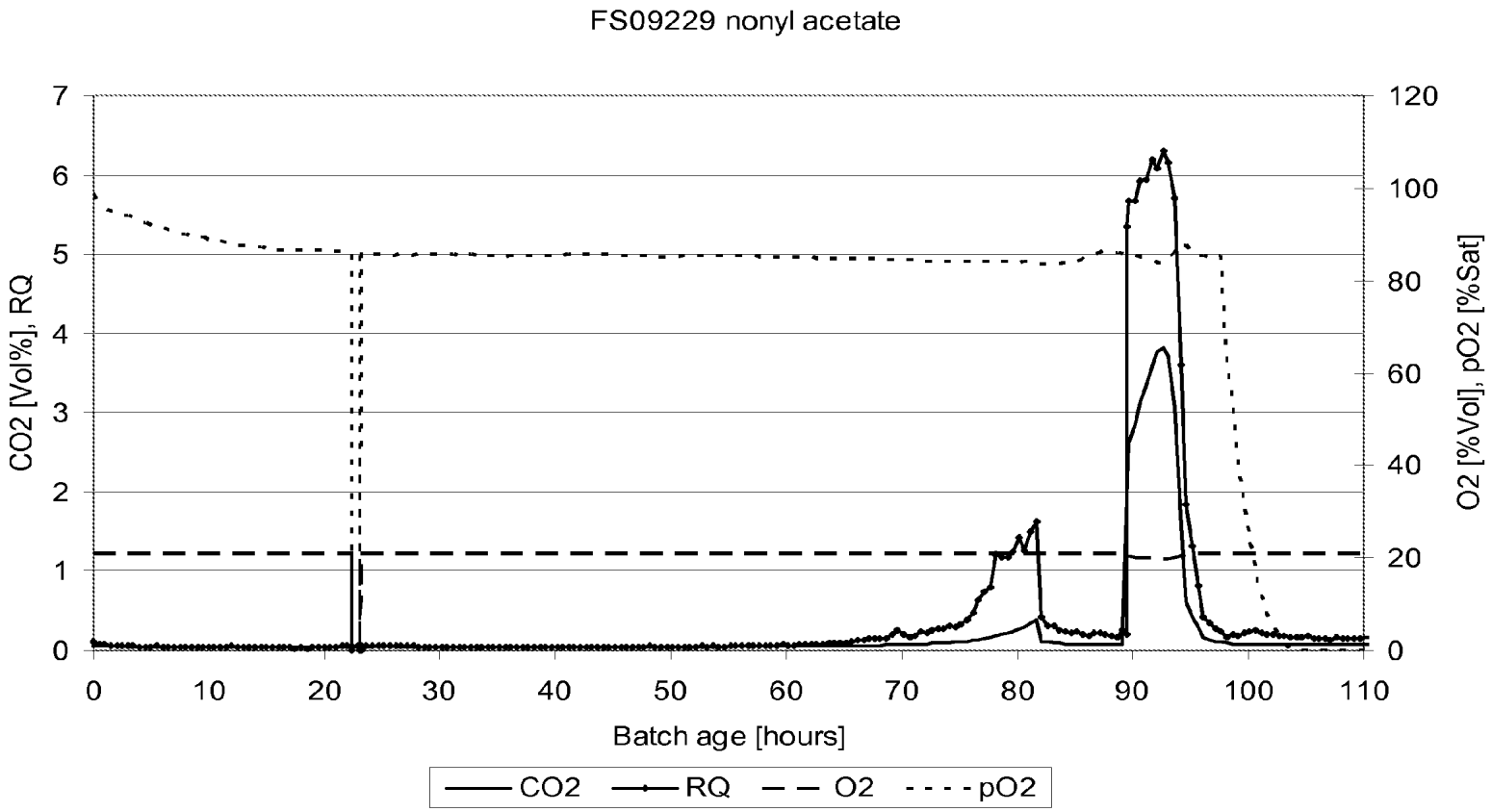

FS09229 nonyl acetate
CO2 [Vol%], RQ
O2 [%Vol], pO2 [%Sat]
Batch age [hours]
0
1
2
3
4
5
6
7
0
20
40
60
80
100
120
0
10
20
30
40
50
60
70
80
90
100
110
CO2
RQ
O2
pO2

Figure 10

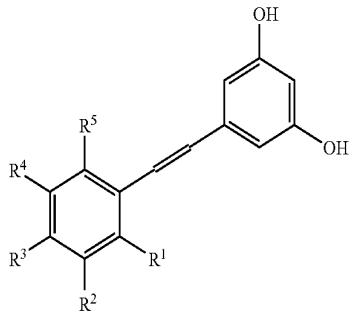
MICROBIAL BIOREACTION PROCESS

FIELD OF THE INVENTION

[0001] This invention relates generally to a bioreactor process in which a stilbenoid (i.e a hydroxystilbene) is produced using a two phase cultivation medium.

BACKGROUND OF THE INVENTION

[0002] There have recently been proposed recombinant micro-organisms that have the capacity to produce certain stilbenoids of the general formula 1:



wherein each of R^1, R^2, R^3, R^4 and R^5 independently is hydrogen or hydroxy. Examples of such compounds include resveratrol (only R^3 being hydroxy) and pinosylvin (all of the R groups being hydrogen), see for instance WO2006/089898.

[0003] EP1181383 describes the in-situ extraction of a micro-organism fermentation product into an encapsulated organic solvent, the purpose being to prevent inhibition of production of the fermentation product caused by the product itself by sequestering it into the encapsulated solvent. This is therefore an approach to address the problem of a poor yield of the desired product.

[0004] U.S. Pat. No. 4,865,973 also tackles the problem of low metabolite yields due to product inhibition, this time by extraction of ethanol during cultivation of *Saccaromyces cerevisiae* yeast into a non-encapsulated solvent such as dodecylacetate.

[0005] US2004/0229326 again tackles the problem of product inhibition, this time in relation to aromatic compounds such as cinnamic acid, using a two phase extractive fermentation based on one or more of several defined solvents which include methyl decanoate.

[0006] Similarly, EP1715032 discloses a two phase fermentation using yeast to produce aroma compounds such as 2-phenylethanol with propylene glycol as extracting solvent to avoid product inhibition.

[0007] In fermentations to produce the stilbenoids with which the invention is concerned there is no problem relating to product inhibition however, as the existing strains of micro-organisms produce these compounds only in very small yields and it is not disclosed that they are secreted into the culture medium. Also, we have found that the solubility limit of the compounds is too low for product inhibition to become a problem.

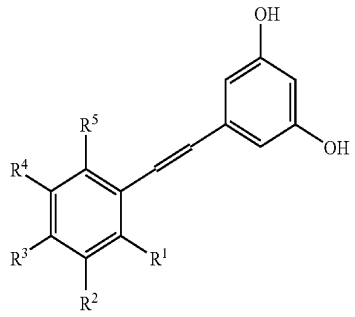
[0008] Teachings such as WO2004/092344 describe biphasic reaction media for carrying out cell free enzymatic or other conversions, but this is of little relevance since there is no exposure of micro-organisms to the biphasic system.

SUMMARY OF THE INVENTION

[0009] Previously described micro-organisms have not been disclosed to release stilbenoids into the culture medium, as opposed to accumulating it within the micro-organism cells, although we have found that strains that we have described can do so. Also, a higher yield of stilbenoid compounds is desirable compared to that released into the culture medium by previously described micro-organisms. Strains of yeast and of other fungi or of bacteria which we have developed are such that the concentration of hydroxystilbene secreted into the medium by the micro-organisms is so high as to reach saturation, leading to precipitation of the product.

[0010] Accordingly, the invention provides in a first aspect a method for the production of a cis- or trans-stilbenoid of the general formula 1:

Formula 1



in which each of R^1, R^2, R^3, R^4 and R^5 independently is hydrogen or hydroxy, or a glycosylated or oligomeric form thereof, comprising cultivating a micro-organism producing said stilbenoid, wherein said cultivation is performed in a multi-phase system comprising at least an aqueous first phase containing said micro-organism and a second phase immiscible with said aqueous phase in which (e.g. as which) said stilbenoid accumulates.

[0011] The second phase in which the stilbenoid accumulates may be composed of the stilbenoid itself. The stilbenoid may then be recovered at least in part simply by separating the accumulated solids from the fermentation, normally followed by further purification steps.

[0012] However, the stilbenoid may also deposit onto vessel walls, stirrer shafts, sensor, baffles and other apparatus constituents. If it is desired to prevent this, the second phase may be a solvent for the stilbenoid which is compatible with the fermentation requirements of the micro-organism. This will prevent accumulating solids disturbing fermentation process control and down-stream processing, desirably preventing precipitation without reduction of the hydroxystilbene yield.

[0013] Preferably, one of said first and second phases is dispersed within the other and preferably the first (aqueous) phase is continuous and the second phase is dispersed.

[0014] Said stilbenoid may be resveratrol (only R^3═OH), pinosylvin (all R groups are hydrogen) or piceatannol (only R^3 and either R^2 or R^4 is OH). Preferably, not more than 3 of the R groups are hydroxy. Preferably, the stilbenoid is trans.

[0015] Said second phase is preferably a liquid. Optionally, said second phase is a micro-encapsulated liquid. Preferably, said liquid or micro-encapsulated liquid comprises or consists of an ester. Said ester is suitably of the general formula R^6—COO—R^7, and R^6 is H or an aliphatic straight or branched chain hydrocarbon moiety of from 1-6 carbon atoms and R^7 is an aliphatic straight or branched chain hydrocarbon moiety of from 2-16 carbon atoms, or a heteroatom containing hydrocarbon moiety of from 2 to 16 carbon atoms or an aromatic or heteroaromatic moiety of from 5 to 16 carbon atoms. R^7 may have from 3 to 9 carbon atoms. R^6 may have from 1 to 4 carbon atoms. Alternatively, R^6 may have from 6 to 12 carbon atoms and R^7 may have from 1 to 6 carbon atoms. For instance, the solvent may be methyl decanoate, propyl decanoate or butyl decanoate or the corresponding undecanoate or dodecanoate esters.

[0016] Preferably, said ester is an octyl acetate, especially n-octyl acetate.

[0017] Optionally, said liquid comprises or further comprises an alkane. It may consist of a said alkane and a said ester.

[0018] Said alkane may be a C_6 to C_{16} straight or branched chain alkane, e.g. a C_{9-14} alkane, e.g. a C_{1-2} alkane. Preferably, said alkane is n-dodecane.

[0019] Preferably, said micro-organism, when cultivated in said aqueous phase without said immiscible phase, is capable of producing said stilbenoid in an amount sufficient to reach a saturated concentration thereof in said aqueous phase and to precipitate therefrom.

[0020] Methods according to the invention may further comprise separating said second phase and extracting said stilbenoid therefrom.

[0021] In a second aspect, the invention provides a method for producing an extraction solvent tolerant micro-organism strain producing a metabolite comprising:

(a) cultivating a starting micro-organism in a multi-phase system comprising at least an aqueous first phase containing said micro-organism and a second phase immiscible with said aqueous phase in which said metabolite accumulates, one of said first and second phases preferably being dispersed within the other, said second phase comprising a first solvent component to which the micro-organism is more tolerant and a first concentration of a second solvent component to which the micro-organism is less tolerant,

(b) recovering progeny micro-organism from said cultivation (a), and

(c) culturing said progeny micro-organism in a said multi-phase system in which the concentration of said second solvent component is increased above said first concentration.

[0022] In a further aspect, the invention provides A method for the production of a cis- or trans-stilbenoid of the general formula 1, comprising cultivating a micro-organism producing said stilbenoid, wherein said cultivation is conducted in a culture medium comprising or consisting of an aqueous phase and produces an amount of said stilbenoid (e.g. resveratrol) released from the micro-organisms into the culture medium which exceeds the solubility limit of said stilbenoid in said aqueous phase.

[0023] The method may be operated such that said stilbenoid precipitates from said culture medium. Alternatively, the cultivation is performed in a said culture medium which is a multi-phase system comprising at least said aqueous phase containing said micro-organism and a liquid solvent immiscible with said aqueous phase in which said stilbenoid accumulates.

[0024] Then it is optional whether said liquid solvent forms a liquid-liquid interface with said aqueous phase or is separated therefrom by encapsulation. Preferably, one of said aqueous phase and said liquid solvent is dispersed within the other and preferably the aqueous phase is continuous and said liquid solvent is dispersed therein.

[0025] All preferred features of the first aspect of the invention also apply in relation to the second aspect and this further aspect also.

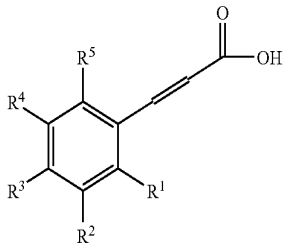
DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

[0026] The micro-organisms used may be naturally occurring, or recombinant micro-organisms, or may be micro-organisms obtained by directed evolution from a starting naturally occurring or recombinant micro-organism. Repeated cultivation of micro-organism cells in a two phase system as described herein will generally produce evolved cells more suited to withstanding the conditions.

[0027] Micro-organisms that may be employed include fungi, including both filamentous fungi and yeasts, and bacteria. Yeasts are preferred, especially strains of *S. cerevisiae.*

[0028] The micro-organism may be one having an operative metabolic pathway comprising at least one enzyme activity, said pathway producing a said stilbenoid or an oligomeric or glycosidically-bound derivative thereof from a precursor aromatic acid of the general formula 2:

Formula 2



wherein each R group is as defined above.

[0029] For instance, the micro-organism may be one producing resveratrol from coumaric acid, producing pinosylvin from cinnamic acid, and/or producing piceatannol from caffeic acid.

[0030] The transformation of the said aromatic acid to the compound of Formula 1 may be by the action of an exogenous stilbene synthase expressed in said micro-organism, usually in conjunction with a suitable aromatic acid-CoA ligase serving to form the CoA thioester of the aromatic acid which together with malonyl-CoA acts as a substrate for the stilbene synthase.

[0031] Stilbene synthases are rather promiscuous enzymes that can accept a variety of physiological and non-physiological substrates. For instance, addition of various phenylpropanoid CoA starter esters led to formation of several products in vitro in Ikuro Abe et al., 2004 and Morita et al., 2001. Likewise it has been shown that resveratrol synthase from rhubarb (*Rheum tartaricum*) indeed synthesized a small amount of pinosylvin when cinnamoyl-CoA was used as substrate instead of coumaroyl-CoA (Samappito et al., 2003).

[0032] Micro-organisms producing resveratrol for use in the invention may be as described in WO2006/089898. In particular, the micro-organism may be one having an operative metabolic pathway comprising at least one enzyme activity, said pathway producing resveratrol, or an oligomeric or glycosidically-bound derivative thereof, from 4-coumaric acid.

[0033] Micro-organisms producing pinosylvin for use in the invention may be as described in WO2008/009728 and therefore may be one that has an operative metabolic pathway comprising at least one enzyme activity, said pathway producing pinosylvin, or an oligomeric or glycosidically-bound derivative thereof, from cinnamic acid.

[0034] Malonyl-CoA for said stilbenoid forming reaction may be produced endogenously.

[0035] The stilbene synthase may be expressed in said micro-organism from nucleic acid coding for said enzyme which is not native to the micro-organism and may be resveratrol synthase (EC 2.3.1.95) from a plant belonging to the genus of *Arachis*, a plant belonging to the genus of Rheum, or a plant belonging to the genus of *Vitus* or any one of the genera *Artocarpus, Clintonia, Morus, Vaccinium, Pinus, Picea, Lilium, Eucalyptus, Parthenocissus, Cissus, Calochortus, Polygonum, Gnetum, Artocarpus, Nothofagus, Phoenix, Festuca, Carex, Veratrum, Bauhinia* or *Pterolobium* or may be a pinosylvin synthase (EC 2.3.1.146) from a plant belonging to the genus of *Pinus*, e.g. *P. sylvestris, P. strobes, P. densiflora, P. taeda*, a plant belonging to the genus *Picea*, or any one of the genus *Eucalyptus*.

[0036] For the preferential production of pinosylvin, the stilbene synthase may be one which exhibits a higher turnover rate with cinnamoyl-CoA as a substrate than it does with 4-coumaroyl-CoA as a substrate, e.g. by a factor of at least 1.5 or at least 2. Thus, in further preferred embodiments, said stilbene synthase is a pinosylvin synthase, suitably from a tree species such as a species of *Pinus, Eucalyptus, Picea* or *Maclura*. In particular, the stilbene synthase may be a pinosylvin synthase (EC 2.3.1.146) from a plant belonging to the genus of *Pinus*, e.g. *P. sylvestris, P. strobes, P. densiflora, P. taeda*, a plant belonging to the genus of *Picea*, or any one of the genus *Eucalyptus*.

[0037] The aromatic acid precursor may be produced in the micro-organism or may be supplied externally thereto, production by the micro-organism generally being preferred. Such aromatic acid precursors are generally producible in the micro-organism from a suitable amino acid precursor by the action of an enzyme such as a phenylalanine ammonia lyase or tyrosine ammonia lyase. The genes for the production of these enzymes may be recombinantly expressed in the micro-organism.

[0038] Thus, in certain preferred embodiments, said L-phenylalanine ammonia lyase is a L-phenylalanine ammonia lyase (EC 4.3.1.5) from a plant or a micro-organism. The plant may belong to the genus of *Arabidopsis*, e.g. *A. thaliana*, a plant belonging to the genus of *Brassica*, e.g. *B. napes, B. rapa*, a plant belonging to the genus of *Citrus*, e.g. *C. reticulata, C. clementines, C. limon*, a plant belonging to the genus of *Phaseolus*, e.g. *P. coccineus, P. vulgaris*, a plant belonging to the genus of *Pinus*, e.g. *P. banksiana, P. monticola, P. pinaster, P. sylvestris, P. taeda*, a plant belonging to the genus of *Populus*, e.g. *P. balsamifera, P. deltoides, P. Canadensis, P. kitakamiensis, P. tremuloides*, a plant belonging to the genus of *Solanum*, e.g. *S. tuberosum*, a plant belonging to the genus of *Prunus*, e.g. *P. avium, P. persica*, a plant belonging to the genus of *Vitus*, e.g. *Vitus vinifera*, a plant belonging to the genus of *Zea*, e.g. *Z. mays* or other plant genera e.g. *Agastache, Ananas, Asparagus, Bromheadia, Bambusa, Beta, Betula, Cucumis, Camellia, Capsicum, Cassia, Catharanthus, Cicer, Citrullus, Coffea, Cucurbita, Cynodon, Daucus, Dendrobium, Dianthus, Digitalis, Dioscorea, Eucalyptus, Gallus, Ginkgo, Glycine, Hordeum, Helianthus, Ipomoea, Lactuca, Lithospermum, Lotus, Lycopersicon, Medicago, Malus, Manihot, Medicago, Mesembryanthemum, Nicotiana, Olea, Oryza, Pisum, Persea, Petroselinum, Phalaenopsis, Phyllostachys, Physcomitrella, Picea, Pyrus, Quercus, Raphanus, Rehmannia, Rubus, Sorghum, Sphenostylis, Stellaria, Stylosanthes, Triticum, Trifolium, Triticum, Vaccinium, Vigna, Zinnia*. The micro-organism might be a fungus belonging to the genus *Agaricus*, e.g. *A. bisporus*, a fungus belonging to the genus *Aspergillus*, e.g. *A. oryzae, A. nidulans, A. fumigatus*, a fungus belonging to the genus *Ustilago*, e.g. *U. maydis*, a bacterium belonging to the genus *Rhodobacter*, e.g. *R. capsulatus*, a bacterium belonging to the genus *Streptomyces*, e.g. *S. maritimus*, a bacterium belonging to the genus *Photorhabdus*, e.g. *P. luminescens*, a yeast belonging to the genus *Rhodotorula*, e.g. *R. rubra*.

[0039] A suitable tyrosine ammonia lyase (EC 4.3.1.5) may be derived from yeast or bacteria. Suitably, the tyrosine ammonia lyase is from the yeast *Rhodotorula rubra* or from the bacterium *Rhodobacter capsulatus*.

[0040] Where the immediate product of the conversion of amino acid to aromatic acid is an aromatic acid that is not suitable as the immediate precursor of the desired stilbenoid, it may be converted to a more appropriate aromatic acid enzymatically by the micro-organism. For instance, cinammic acid may be converted to coumaric acid by a cinnamate-4-hydroxylase (C4H). Thus, said 4-coumaric acid may be produced from trans-cinnamic acid by a cinnamate 4-hydroxylase, which preferably is expressed in said micro-organism from nucleic acid coding for said enzyme which is not native to the micro-organism.

[0041] In certain preferred embodiments, said cinnamate-4-hydroxylase is a cinnamate-4-hydroxylase (EC 1.14.13.11) from a plant or a micro-organism. The plant may belong to the genus of *Arabidopsis*, e.g. *A. thaliana*, a plant belonging to the genus of *Citrus*, e.g. *C. sinensis, C.×paradisi*, a plant belonging to the genus of *Phaseolus*, e.g. *P. vulgaris*, a plant belonging to the genus of *Pinus*, e.g. *P. taeda*, a plant belonging to the genus of *Populus*, e.g. *P. deltoides, P. tremuloides, P. trichocarpa*, a plant belonging to the genus of *Solanum*, e.g. *S. tuberosum*, a plant belonging to the genus of *Vitus*, e.g. *Vitus vinifera*, a plant belonging to the genus of *Zea*, e.g. *Z. mays*, or other plant genera e.g. *Ammi, Avicennia, Camellia, Camptotheca, Catharanthus, Glycine, Helianthus, Lotus, Mesembryanthemum, Physcomitrella, Ruta, Saccharum, Vigna*. The micro-organism might be a fungus belonging to the genus *Aspergillus*, e.g. *A. oryzae*.

[0042] The conversion of the aromatic acid precursor into its CoA derivative may be performed by a suitable endogenous or recombinantly expressed enzyme. Both cinnamoyl-CoA and coumaroyl-CoA may be formed in a reaction catalysed by an enzyme in which ATP and CoA are substrates and ADP is a product by a 4-coumarate-CoA ligase (also referred to as 4-coumaroyl-CoA ligase). Known 4-coumarate-CoA ligase enzymes accept either 4-coumaric acid or cinnamic acid as substrates and produce the corresponding CoA derivatives. Generally, such enzymes are known as '4-coumarate-CoA ligase' whether they show higher activity with 4-coumaric acid as substrate or with cinnamic acid as substrate. However, we refer here to enzymes having that substrate preference as 'cinnamate-CoA ligase' enzymes (or cinnamoyl-CoA-ligase). One such enzyme is described for instance in Aneko et al., 2003.

[0043] Said 4-coumarate-CoA ligase or cinnamate-CoA ligase may be a 4-coumarate-CoA ligase/cinnamate-CoA ligase (EC 6.2.1.12) from a plant, a micro-organism or a nematode. The plant may belong to the genus of *Abies*, e.g. *A. beshanzuensis, B. firma, B. holophylla*, a plant belonging to the genus of *Arabidopsis*, e.g. *A. thaliana*, a plant belonging to the genus of *Brassica*, e.g. *B. napes, B. rapa, B. oleracea*, a plant belonging to the genus of *Citrus*, e.g. *C. sinensis*, a plant belonging to the genus of *Larix*, e.g. *L. decidua, L. gmelinii, L. griffithiana, L. himalaica, L. kaempferi, L. laricina, L. mastersiana, L. occidentalis, L. potaninii, L. sibirica, L. speciosa*, a plant belonging to the genus of *Phaseolus*, e.g. *P. acutifolius, P. coccineus*, a plant belonging to the genus of *Pinus*, e.g. *P. armandii P. banksiana, P. pinaster*, a plant belonging to the genus of *Populus*, e.g. P. balsamifera, *P. tomentosa, P. tremuloides*, a plant belonging to the genus of *Solanum*, e.g. *S. tuberosum*, a plant belonging to the genus of *Vitus*, e.g. *Vitus vinifera*, a plant belonging to the genus of *Zea*, e.g. *Z. mays*, or other plant genera e.g. *Agastache, Amorpha, Cathaya, Cedrus, Crocus, Festuca, Glycine, Juglans, Keteleeria, Lithospermum, Lolium, Lotus, Lycopersicon, Malus, Medicago, Mesembryanthemum, Nicotiana, Nothotsuga, Oryza, Pelargonium, Petroselinum, Physcomitrella, Picea, Prunus, Pseudolarix, Pseudotsuga, Rosa, Rubus, Ryza, Saccharum, Suaeda, Thellungiella, Triticum, Tsuga*. The micro-organism might be a filamentous fungi belonging to the genus *Aspergillus*, e.g. *A. flavus, A. nidulans, A. oryzae, A. fumigatus*, a filamentous fungus belonging to the genus *Neurospora*, e.g. *N. crassa*, a fungus belonging to the genus *Yarrowia*, e.g. *Y. lipolytica*, a fungus belonging to the genus of *Mycosphaerella*, e.g. *M. graminicola*, a bacterium belonging to the genus of *Mycobacterium*, e.g. *M. bovis, M. leprae, M. tuberculosis*, a bacterium belonging to the genus of *Neisseria*, e.g. *N. meningitidis*, a bacterium belonging to the genus of *Streptomyces*, e.g. *S. coelicolor*, a bacterium belonging to the genus of *Rhodobacter*, e.g. *R. capsulatus*, a nematode belonging to the genus *Ancylostoma*, e.g. *A. ceylanicum*, a nematode belonging to the genus *Caenorhabditis*, e.g. *C. elegans*, a nematode belonging to the genus *Haemonchus*, e.g. *H. contortus*, a nematode belonging to the genus *Lumbricus*, e.g. *L. rubellas*, a nematode belonging to the genus *Meilodogyne*, e.g. *M. hapla*, a nematode belonging to the genus *Strongyloidus*, e.g. *S. rattii, S. stercoralis*, a nematode belonging to the genus *Pristionchus*, e.g. *P. pacificus*.

[0044] Optionally, one may express, over express, or recombinantly express in said organism an NADPH:cytochrome P450 reductase (CPR). This may be a plant CPR. Alternatively, a native NADPH:cytochrome P450 reductase (CPR) may be overexpressed in said micro-organism. Optionally, said NADPH:cytochrome P450 reductase is a NADPH:cytochrome P450 reductase (EC 1.6.2.4) from a plant belonging to the genus of *Arabidopsis*, e.g. *A. thaliana*, a plant belonging to the genus of *Citrus*, e.g. *C. sinensis, C.×paradisi*, a plant belonging to the genus of *Phaseolus*, e.g. *P. vulgaris*, a plant belonging to the genus of *Pinus*, e.g. *P. taeda*, a plant belonging to the genus of *Populus*, e.g. *P. deltoides, P. tremuloides, P. trichocarpa*, a plant belonging to the genus of *Solanum*, e.g. *S. tuberosum*, a plant belonging to the genus of *Vitus*, e.g. *Vitus vinifera*, a plant belonging to the genus of *Zea*, e.g. *Z. mays*, or other plant genera e.g. *Ammi, Avicennia, Camellia, Camptotheca, Catharanthus, Glycine, Helianthus, Lotus, Mesembryanthemum, Physcomitrella, Ruta, Saccharum, Vigna*.

[0045] Because, as described above, for the production of pinosylvin, production of cinnamic acid by a PAL enzyme and also its conversion on to pinosylvin is preferred to either the production of coumaric acid from tyrosine by a substrate promiscuous PAL or by conversion of cinnamic acid by a C4H enzyme, micro-organisms for use in the invention to produce pinosylvin preferably have a PAL which favours phenylalanine as a substrate (thus producing cinnamic acid) over tyrosine (from which it would produce coumaric acid). Preferably, therefore, the ratio K_m(phenylalanine)/K_m(tyrosine) for the PAL is less than 1:1, preferably less 1:5, e.g. less than 1:10. As usual, K_m is the molar concentration of the substrate (phenylalanine or tyrosine respectively) that produces half the maximal rate of product formation (V_{max}).

[0046] Except where micro-encapsulation is used, in choosing a solvent to act as the water immiscible phase in the cultivation, there generally will be some tension between opposing requirements for a solvent that does not hamper the growth of the micro-organism and one that successfully extracts the hydroxystilbene product. Generally, the less miscible with water is the solvent, the less it will interfere with the micro-organism growth, but the less effective it will be in extracting the hydroxystilbene.

[0047] The rate at which the system is agitated will also have an effect, greater agitation tending to increase the interference of the solvent with the growth of the micro-organism. The toxicity of the water immiscible material for the micro-organism may therefore be regarded as being divided into a direct toxic effect due to the concentration of the immiscible solvent in the aqueous medium and a phase toxicity due to the physical presence of the immiscible phase which can exert effects by nutrient extraction, limited access to nutrients due to emulsion formation, cell coating, attraction to the interface and, the most detrimental effect, disruption of cell membranes.

[0048] The rate of agitation employed should therefore be balanced against the nature of the immiscible solvent material.

[0049] However, optionally, the solvent is physically separated from the aqueous phase by micro-encapsulation, as described in Stark et al, 2003 and EP1181383 using for instance solvent filled alginate micro-spheres of for instance 1-4 mm diameter. The encapsulating material is chosen to be permeable to the stilbenoid. This can prevent phase toxicity, although the aqueous phase may remain saturated with the solvent. This allows a more free choice of solvent on the basis of its extractive power for the stilbenoid having regard to the stilbenoid solubility therein and its partition coefficient for the stilbenoid.

[0050] The solvent is preferably one in which the micro-organism of interest, if necessary after adaptation as described in Example 12, is able to grow in an aqueous phase culture medium in liquid-liquid interface contact with the said solvent at a growth rate of at least 50% of the growth rate obtainable in the aqueous phase culture medium without the solvent being present. Such a solvent may be referred to as a biocompatible solvent.

[0051] Preferred solvents have a partition coefficient logP between water and octanol of not more than 4.4, preferably not more than 4.0. Such solvents are generally suitable for all of the stilbenoids. However, for the most hydrophobic stilbenoids, especially pinosylvin, a logP value of up to 7.5 may be used, e.g. up to 6.5.

[0052] Having regard particularly for their extraction affinity for more polar hydroxy stilbenes, preferred solvents are esters, especially esters of the general formula R^6—COO—R^7, where R^6 is H or an optionally substituted aliphatic

straight or branched chain hydrocarbon moiety of from 1-6 carbon atoms, or an optionally substituted aromatic or heteroaromatic moiety of from 5 to 6 carbon atoms, and R^7 is an optionally substituted aliphatic straight or branched chain hydrocarbon moiety of from 2-16 carbon atoms, or an optionally substituted heteroatom containing hydrocarbon moiety of from 2 to 16 carbon atoms or an optionally substituted aromatic or heteroaromatic moiety of from 5 to 16 carbon atoms. Suitably, R^7 has from 3 to 9 carbon atoms. Suitably the ester is formed between a short chain acid and a long chain alcohol, e.g. where R^6 has from 1 to 4 carbon atoms and R^7 has from 6 to 12 carbon atoms.

[0053] Said ester is preferably an octyl acetate, e.g. n-octyl acetate (logP=3.7). Alternatives include hexyl, heptyl, nonyl (logP=4.2) and decyl acetates, and the corresponding formates and propionates.

[0054] Alternatively, esters formed between long chain acids and short chain alcohols may be used, e.g. where R^6 may have from 6 to 12 carbon atoms and R^9 may have from 1 to 6 carbon atoms. For instance, the solvent may be methyl decanoate (logP=4.3), propyl decanoate or butyl decanoate or the corresponding undecanoate or dodecanoate esters.

[0055] Also, long chain ketones such as a C_8 to C_{12} ketones, e.g. undecanone may be used. These may be of the formula R^8COR^9 where R^8 and R^9 independently may be an optionally substituted aliphatic straight or branched chain hydrocarbon moiety, e.g. where R^8 is C_{1-5}, more preferably C_{1-3}, and R^9 is C_{7-12}.

[0056] Other suitable solvents may be as described in U.S. Pat. No. 4,865,973. Except where these overlap with those described above however they are in general less preferred. They include double bond unsaturated aliphatic alcohols having 12 or more carbon atoms, saturated branched chain aliphatic alcohols having 14 or more carbon atoms or mixtures thereof (e.g. guerbet alcohols), double bond unsaturated aliphatic acids having 12 or more carbon atoms, aliphatic and aromatic mono- di- or tri-esters having 12 or more carbon atoms, aliphatic noncyclic ketones and aliphatic aldehydes having 12 or more carbon atoms.

Examples include:

oleyl alcohol, (cis-9-octadecen-1-ol), phytol (3,7,11,15-tetramethyl-2-hexadecen-1-ol), isophytol (3,7,11,15-tetramethyl-1-hexadecen-3-ol), isostearyl alcohol e.g. the commercial product sold under the trademark ADOL 66, isocetyl alcohol e.g. the commercial product sold under the trademark Eutanol G-16, octyl dodecanol e.g. the commercial product sold under the trademark Eutanol G, oleic acid (cis-9-octadecenoic acid), linoleic acid, (9,11-octadecadienoic acid), ricinoleic acid, (12-hydroxy-9-octadecenoic acid), dodecyl acetate (CH_3 $COO(CH_2)_{11}$), butyl dodecanoate, (CH_3 $(CH_2)_{10}$ $COOC_4H_9$), dibutyl sebacate (C_4H_9 $OOC(CH_2)_8H_{17}COOC_4H_9$, di (2-ethylhexyl)sebacate, ($C_8H_{17}OOC(CH_2)_8$ $COOC_8H_{17}$), dibutyl adipate (C_4H_9 $OOC(CH_2)_4$ $COOC_4H_9$), di(2-ethylhexyl)adipate, (C_8H_{17} $OOC(CH_2)_4$ $COOC_8H_{17}$), di(2-ethylhexyl)phthalate, ($C_8H_{17}OOCC_6H_4COOC_8H_{17}$), di(3,5,5-trimethyhexyl), phthalate ($C_8H_{17}OOCC_6H_4COOC_8H_{17}$), glyceroltrideca-([$CH_3$ $(CH_2)_8COOCH_2]_2$ $CHOCO(CH_2)_8CH_3$)noate, 2-dodecanone ($CH_3CO(CH_2)_9CH_3$), dodecanal ($CH_3(CH_2)$ 10 CHO). the commercial product sold under the trademark ADOL 85 NF (69 percent oleyl alcohol), the commercial product sold under the trademark ADOL 330 (62 percent oleyl alcohol), and the commercial product sold under the trademark HD oleyl alcohol (commercial oleyl alcohol).

[0057] Generally, all the solvents described above may be used in any admixture with one or more others.

[0058] For the production of pinosylvin, which is the least polar of the hydroxystilbenes of formula 1, an alkane may be used. This may be a C_6 to C_{16} (e.g. C_9 to C_{14}) straight or branched chain alkane such as a nonane, decane, undecane, dodecane or higher, e.g. n-dodecane. However, used by itself, n-dodecane does not have sufficient polarity to be a good extractant for resveratrol and more hydroxylated compounds.

[0059] On the other hand, the esters are less well tolerated by certain micro-organisms as regards toxicity and in some cases can steer hydroxystilbene production towards resveratrol and away from pinosylvin.

[0060] For the mixed production of resveratrol and pinosylvin for instance, it is therefore preferred to use a mixture of a said ester and a said alkane, e.g. octyl acetate and n-dodecane.

[0061] Micro-organisms that do not fully tolerate a particular solvent such as octyl acetate may be evolved to do so by methods described briefly above.

[0062] In the present context the term "micro-organism" relates to microscopic organisms, including bacteria, microscopic fungi, including yeast. More specifically, the micro-organism may be a fungus, and more specifically a filamentous fungus belonging to the genus of *Aspergillus*, e.g. *A. niger, A. awamori, A. oryzae, A. nidulans*, a yeast belonging to the genus of *Saccharomyces*, e.g. *S. cerevisiae, S. kluyveri, S. bayanus, S. exiguus, S. sevazzi, S. uvarum*, a yeast belonging to the genus *Kluyveromyces*, e.g. *K. lactis K. marxianus* var. *marxianus, K. thermotolerans*, a yeast belonging to the genus *Candida*, e.g. *C. utilis C. tropicalis, C. albicans, C. lipolytica, C. versatilis*, a yeast belonging to the genus *Pichia*, e.g. *P. stipidis, P. pastoris, P. sorbitophila*, or other yeast genera, e.g. *Cryptococcus, Debaromyces, Hansenula, Pichia, Yarrowia, Zygosaccharomyces* or *Schizosaccharomyces*. Concerning other micro-organisms a non-exhaustive list of suitable filamentous fungi is: a species belonging to the genus *Penicillium, Rhizopus, Fusarium, Fusidium, Gibberella, Mucor, Mortierella*, and *Trichoderma*.

[0063] Concerning bacteria a non-exhaustive list of suitable bacteria is follows: a species belonging to the genus *Bacillus*, a species belonging to the genus *Escherichia*, a species belonging to the genus *Lactobacillus*, a species belonging to the genus *Lactococcus*, a species belonging to the genus *Corynebacterium*, a species belonging to the genus *Acetobacter*, a species belonging to the genus *Acinetobacter*, a species belonging to the genus *Pseudomonas*, etc.

[0064] The preferred micro-organisms of the invention may be *S. cerevisiae, A. niger, A. nidulans, A. oryzae, E. coli, L. lactis* or *B. subtilis*.

[0065] The constructed and engineered micro-organism can be cultivated using commonly known processes, including chemostat, batch, fed-batch cultivations, etc.

[0066] Stilbenoids produced as described herein may optionally be isolated or purified and suitable methods include solvent extraction with n-hexane, followed by sequential extraction with 100% ether, acetone, methanol and water, and chromatographic purification on a silicagel column using a n-hexane/ethyl acetate (2/1) system.

[0067] The micro-organism may be fed with a carbon substrate which is optionally selected from the group of fermentable carbon substrates consisting of monosaccharides, oligosaccharides and polysaccharides, e.g. glucose, fructose, galactose, xylose, arabinose, mannose, sucrose, lactose,

erythrose, threose, and/or ribose. Said carbon substrate may additionally or alternatively be selected from the group of non-fermentable carbon substrates including ethanol, acetate, glycerol, and/or lactate. Said non-fermentable carbon substrate may additionally or alternatively be selected from the group of amino acids and may be phenylalanine and/or tyrosine.

BRIEF DESCRIPTION OF THE DRAWINGS

[0068] To assist in the ready understanding of the above description of the invention reference has been made to the accompanying drawings in which are shown:

[0069] FIG. **1**A: Fermentation profile for PALCPR-control (see Example 3): CO_2-evolution (CO2 Vol %), O_2 consumption (O_2 Vol %) and dissolved oxygen (PO_2% Sat) are shown.

[0070] FIG. **1**B: Fermentation profile for PALCPR-solvent (see Example 3): CO_2-evolution (CO2 Vol %), O_2 consumption (O_2 Vol %) and dissolved oxygen (PO_2% Sat) are shown.

[0071] FIG. **2**: Fermentation profile for PALCPR-evolved-I (Example 4): CO_2-evolution (CO2 Vol %), O_2 consumption (O_2 Vol %) and dissolved oxygen (PO_2% Sat) are shown.

[0072] FIG. **3**: Fermentation profile for PALCPR-evolved-II (Example 5): CO_2-evolution (CO2 Vol %), O_2 consumption (O_2 Vol %) and dissolved oxygen (PO_2% Sat) are shown.

[0073] FIG. **4**: Fermentation profile for PALCPR-evolved-III (Example 6): CO_2-evolution (CO2 Vol %), O_2 consumption (O_2 Vol %) and dissolved oxygen (PO_2% Sat) are shown.

[0074] FIG. **5**: Principle of two-phase fermentation. A: extraction of stilbenoids into solvent phase with low stirring and hence physical separate phases; B: extraction of stilbenoids into solvent phase with high stirring and hence with mixed phases.

[0075] FIG. **6**: Structure of the fused divergent TEF1-TDH3 promoters referred to in Example 9.

[0076] FIG. **7**: Structure of a plasmid vector pESC-HIS-4CL-VST1 containing galactose inducible promotors Gal1/Gal10 referred to in Example 9 (VII).

[0077] FIG. **8**: Structure of a plasmid vector pESC-HIS-TDH3-4CL-TEF-VST1 referred to in Example 9(VII).

[0078] FIG. **9**: History plot of parameters of cultivation measured in Example 10.

[0079] FIG. **10**: History plot of parameters of cultivation measured in Example 11.

EXAMPLES

Example 1

Expression of the PAL-Pathway to Resveratrol in *S. cerevisiae* Strain Overexpressing Native *S. cerevisiae* NADP-Cytochrome P450 Reductase (CPR)

[0080] A yeast strain FSSC-PAL2C4H4CL2VST1-pADH1CPR1 was used. This is a strain of *S. cerevisiae* having introduced therein genes expressing PAL2 from *A. thaliana*, C4H from *A. thaliana,* 4CL2 from *A. thaliana*, and VST1 from *Vitis vinifera* with overexpressed CPR from *A. thaliana* (see WO2006/089898 and WO2008/009728 for details). In summary construction of the strain is described further below and is divided in parts A, B, C, D and E.

A: Construction of a strain overexpressing native *S. cerevisiae* NADP-cytochrome P450 reductase (CPR).

[0081] The native promoter of *S. cerevisiae* NADP-cytochrome P450 reductase CPR1 gene (encoded by YHR042W) was replaced with the constitutive *S. cerevisiae* alcohol dehydrogenase ADH1 promoter via chromosomal promoter exchange using the "bi-partite" PCR-based allele replacement method. Primers A and B were used to generate fragment CPR1-UP (Table 1) via PCR at a melting temperature of 56° C. using *S. cerevisiae* genomic DNA as template. Primers C and D were then used to generate fragment CPR1-S via PCR at a melting temperature of 56° C. using *S. cerevisiae* genomic DNA as template. Fragments AD1 (klURA 3' end fused to promoter ADH1) and AD2 (promoter ADH1 fused to KlURA 5' end) were generated via PCR using primers AD-fw and Int3' and IntS' and AD-rv at a melting temperature of 56° C. and 56° C., respectively. Plasmid pWAD1 was used as template for generation of fragment AD1 and plasmid pWAD2 was used for generating fragment AD2.

[0082] Fragments CPR_UP were then fused to fragment AD2 using fusion PCR with primers A and Int3' at a melting temperature of 56° C. resulting in fusion fragment 1 (bi-partite substrate 1). A second fusion PCR was used to fuse fragments AD1 and CPR-S with IntS' and primer D at a melting temperature of 56° C. resulting in fusion fragment 2 (bi-partite substrate 2).

[0083] Fusion fragments 1 and 2 (bi-partite substrates 1 and 2) were purified on agarose gel and used for co-transformation of *S. cerevisiae* strain FS01528 (Mata, ura3 his 3) and the transformants were plated on SC-URA plates and incubated for 2-4 days at 30° C. Transformants were streak purified on SC-ura plates and incubated another 2 days at 30° C. and then plated onto 5-FOA (5-fluoroorotic acid) plates. After incubation for 2 days at 30° C. "pop-out" colonies appeared, which were streak purified on a new 5-FOA-plate and incubated another 2 days at 30° C. and then finally transferred to a rich medium plate YPD. The resulting colonies were analyzed for the presence of fragment of size 1700-1800 base pairs using yeast colony PCR with primers A and AD-rev and a melting temperature at 55° C. and an elongation time of 1 minute and 45 seconds. One of the positive colonies from the colony PCR containing the new replaced ADH1 promoter in front of the CPR1 gene was named FSpADH1-CPR (Mata ura3 his3 pADH1-CPR1) strain.

TABLE 1

Primers and fragments used in the "bi-partite" PCR-based allele replacement method to exchange native *S. cerevisie* CPR1 promoter with *S. cerevisiae* ADH1 promoter

Primers	
A	5'-GTATTCTATATCCACGCCTGCAAAC 3' *1
B	5'-AGTACATACAGGGAACGTCCCTACAGGAACGCAAACTTAAGCTAC 3' *2
C	5'-GCATACAATCAACTATCTCATATACAATGCCGTTTGGAATAGACAACACC 3' *3
D	5'-GCTTCCGCATTACAAATAAAGTCTTCAA 3' *4
AD-fw	5'-GGACGTTCCCTGTATGTACTAGGGGGATCGAAGAAATGATGG 3' *5

TABLE 1-continued

Primers and fragments used in the "bi-partite" PCR-based allele replacement method to exchange native *S. cerevisie* CPR1 promoter with *S. cerevisiae* ADH1 promoter

Int3' 5'-GAGCAATGAACCCAATAACGAAATC 3' *6
Int5' 5'-CTTGACGTTCGTTCGACTGATGAGC 3' *7
AD-rv 5'-TGTATATGAGATAGTTGATTGTATGC 3' *8

Fragments

CPR-UP generated from primers A and B (CPR1 gene fragment upstream of start codon (ATG))
CPR-S generated from primers C and D (CPR1 gene fragment containing start codon (ATG))
AD1 (ADH1 promoter coupled to two thirds of *K.lactis* URA3 towards the 5' end generated from primers AD-fw and Int3')
AD2 (Two thirds of *K.lactis* URA3 towards the 3' end coupled to the ADH1 promoter. Generated from primers Int5' and AD-rv)
Fusion fragment 1 (CPR-UP fragment fused to AD2 fragment)
Fusion fragment 2 (AD1 fragment fused to CPR-S fragment)

*1 SEQ ID NO: 1
*2 SEQ ID NO: 2
*3 SEQ ID NO: 3
*4 SEQ ID NO: 4
*5 SEQ ID NO: 5
*6 SEQ ID NO: 6
*7 SEQ ID NO: 7
*8 SEQ ID NO: 8

B: Isolation of genes encoding PAL, C4H, 4CL, and VST1

[0084] Phenylalanine ammonia lyase (PAL2), cinnamate 4-hydroxylase (C4H), 4-coumarate:CoenzymeA ligase (4CL1) were isolated as described previously (WO2006/089898) via PCR from *A. thaliana* cDNA (BioCat, Heidelberg, Germany).

4-coumarate:CoenzymeA ligase (4CL2) (see WO2006/089898 and PCT/EP2007/057484 for details) was isolated via PCR from *A. thaliana* cDNA (BioCat, Heidelberg, Germany) using the forward primer 5'-GCGAATTCTTATGACGACACAAGATGTGATAGTCAATGAT-3' SEQ ID NO: 9 containing an EcoR1 restriction site and reverse primer 5'-GCACTAGTATCCTAGTTCATTAATCCATTTGCTAGT-CTTGCT-3' SEQ ID NO:10 containing a Spe1 restriction site.

[0085] The VST1 gene encoding *Vitis vinifera* (grapevine) resveratrol synthase (Hain et al, 1993) was synthesized by GenScript Corporation (Piscataway, N.J.). The amino acid sequence (see WO2006/089898 and WO2008/009728 for details) was used as template to generate a synthetic gene optimized for expression in *S. cerevisiae*. The synthetic VST1 gene was delivered inserted in *E. coli* pUC57 vector flanked by BamH1 and Xho1 restriction sites. The synthetic gene was purified from the pUC57 vector by BamH1/Xho1 restriction and purified from agarose gel using the QiaQuick Gel Extraction Kit (Qiagen).

C: Construction of a yeast vector for expression of PAL and C4H

[0086] Plasmid, pESC-URA-PAL-C4H, containing the genes encoding PAL and C4H under the control of the divergent GAL1/GAL10 promoter was constructed as described in Example 3 of WO2006/089898.

D: Construction of a yeast vector for expression of 4CL

[0087] The gene encoding 4CL1 and 4CL2 were isolated as described in previously. The amplified 4CL1 PCR-product was digested with Xba1/BamH1 and ligated into Spe1/BglII digested pESC-TRP vector (Stratagene), resulting in vector pESC-TRP-4CL. The amplified 4CL2 PCR-product was digested with EcoR1/Spe1 and ligated into EcoR1/Spe1 digested pESC-HIS vector (Stratagene), resulting in vector pESC-HIS-4CL2. Two different clones of pESC-TRP-4CL1 and pESC-HIS-4CL2 were sequenced to verify the sequence of the cloned gene.

E: Construction of yeast vectors for expression of 4CL and VST

[0088] The gene encoding VST from *Vitis vinifera* (grape) was isolated as described previously. The purified BamH1/Xho1 digested VST gene fragment was ligated into BamH1/Xho1 digested pESC-HIS-4CL2 plasmid or pESC-trp-4CL1 plasmid (example 15). The resulting plasmids, pESC-HIS-4CL2-VST and pESC-TRP-4CL1-VST contained the genes encoding 4CL1, 4CL2 and VST under the control of the divergent GAL1/GAL10 promoter. The sequence of the gene encoding VST was verified by sequencing of two different clones of pESC-HIS-4CL2-VST and pESC-TRP-4CL1-VST.

[0089] FSpADH1-CPR (Mata ura3 his3 pADH1-CPR1) as described previously was co-transformed with the vectors pESC-URA-PAL-C4H and pESC-HIS-4CL2-VST, resulting in the strain FSSC-PALC4H$_4$CL2VST-pADH1CPR1 (Mata ura3 his3 pADH1-CPR1, pESC-URA-PAL-C4H, pESC-HIS-4CL2-VST).

Example 2

Adaptation of Strain PALCPR to the Presence of Solvents

[0090] Yeast strain FSSC-PAL2C4H4CL2VST1-pADH1CPR1 as described in the previous example was subjected to a train of batch fermentations in a fermentor from Applikon containing defined medium according to Verduyn et al. (1992), containing: 10.0 g/L $(NH_4)_2SO_4$; 3.0 g/L

KH_2PO_4; 0.5 g/L $MgSO_4$.7H2O; trace metals and vitamins with the aim of adapting it to the presence of solvents. The working volume was 1 L and the gas flowrate was set at 1.5 l/m, temperature was uncontrolled and pH was set at 5.5. The first fermentation contained 10 g/l glucose and 50 g/l galactose, and stirring rate was kept low to prevent mixing of the medium- and solvent phase, as illustrated in FIG. **5**A in which is seen a fermentor vessel 10 containing the two separated phases 12 (aqueous) and 14 (solvent) agitated by a stirrer 16 at below 200 rpm. After consumption of the glucose and at the onset of galactose consumption, a mixture of 10 ml octylacetate and 40 ml dodecane was added. Consumption of galactose continued and the strain was harvested and used for a next fermentation. The next fermentation was performed with said harvested strain using similar fermentation conditions as to the previous one, but now with addition of a mixture of 50 ml octylacetate and 100 ml dodecane. Indeed the cells were able to grow in the presence of said solvent mixture, and the strain was harvested and used for a next fermentation. The next fermentation was performed with said harvested strain, using similar conditions as to the previous one, but now the stirring rate was increased to 1000 rpm halfway through the consumption of galactose, causing mixing of the solvent phase with the medium phase as illustrated in FIG. **5**B. Indeed the cells remained able to grow when the phases were mixed and the strain was harvested and used for a next fermentation. The next fermentation was performed with said harvested strain using similar conditions as to the previous one, but now with addition of a mixture of 100 ml octylacetate and 100 ml dodecane. The increase of the octylacetate to 100 ml caused the arrest of cell growth, indicated by a halt in galactose consumption, and therefore the amount of dodecane was increased to 200 ml, in order to further obscure the toxic effect of octylacetate. Indeed the cells were now able to grow in the presence of said solvent mixture at high stirring rate. The strain was harvested and stored in 15% glycerol at −80° C.

Example 3

Determination of Intracellular and Extracellular Levels of Stilbenoids in a Batch Culture of Strain PALCPR

[0091] The last harvested strain as described in previous example was grown in two independent batch cultures with a working volume of 1 liter, containing defined medium according to Verduyn et al. (1992), containing: 10.0 g/L $(NH_4)_2SO_4$; 3.0 g/L KH_2PO_4; 0.5 g/L $MgSO_4.7H_2O$; trace metals and vitamins and 10 g/l glucose and 100 g/l galactose as the carbon sources. Antifoam (300 μl/L, Sigma A-8436) was added to avoid foaming. The carbon source was autoclaved separately from the mineral medium and afterwards added to the fermentor. In addition, the vitamin and trace metal solutions were added to the fermentor by sterile filtration following autoclaving and cooling of the medium. The fermentor system was from Sartorius BBI systems and consisted of a baffled 3-liter reactor vessel with 1 liter working volume equipped with Biostat B Plus controller. The reactor vessel was equipped with one lower-fitted Rushton turbine which was rotating eventually at 1000 rpm, the temperature was kept at 30±1° C., and the pH was kept at 5.5±0.2 by automatic addition of 2M KOH. The gasflow was controlled by a mass flow controller and was set to 1.5 vvm (1.5 l/min). The off-gas was led through a cooled condenser, and was analyzed for O_2 and CO_2 (Model 1308, Innova, Denmark). The initial batch cultures were started by inoculation of the medium with a pre-grown strain that was harvested in the exponential phase and stored at −80° C. in 15% glycerol. The cells were allowed to fully consume the glucose at a stirring speed of 1000 rpm, and the average dissolved oxygen content was kept above 70% of saturated air. After approximately 20 hrs, at the onset of galactose consumption, the stirring rate was turned down to 300 rpm, and to one culture a mixture of 200 ml dodecane and 100 ml of octylacetate was slowly added. This cultivation will further be referred to as PALCPR-solvent, whereas the cultivation without addition of solvent will be referred to as PALCPR-control.

[0092] Upon addition of the solvent mixture to the PALCPR-solvent culture the CO_2 production halted temporarily, but recovered again after 5 minutes. Moreover, the CO_2-production in the PALCPR-control culture also ceased temporarily and recovered soon, indicating that this was more the result of the hampered oxygen transfer imposed by the lowered stirrer speed. The stirrer speed was increased in steps of 100 rpm to 1000 rpm over a period of 5 hours. In order to enable comparison of production of stilbenoids in both cultures, the PALCPR-control culture was subjected to the same conditioning regime. In both cultures the cells kept growing exponentially with concomitant production of CO_2.

[0093] The cells in the PALCPR-control culture consumed the galactose in 17 hours, whereas the galactose in the PALCPR-solvent culture was depleted after a little less than 25 hours. Based upon the CO_2 production the specific growth rate was estimated to be 0.23 1/h for the cells in the PALCPR-control culture, and 0.13 1/h for the cells in the PALCPR-solvent culture (FIGS. **1**A and **1**B). The final biomass content was 27.5 g/l in the PALCPR-control culture and 19.6 g/l in the PALCPR-solvent culture.

[0094] For the determination of stilbenoids, samples were taken at the point of galactose depletion. For the PALCPR-solvent culture, an aliquot of 25 ml of cell broth was collected, and phase separation was initiated by centrifugation at 3500 g for 5 minutes. Both the upper octylacetate phase and the lower aquous medium phase were collected separately with a pipet and directly analyzed for their content of stilbenoids and intermediates by HPLC as follows:

[0095] For quantitative analysis of cinnamic acid, coumaric acid, resveratrol and pinosylvin, samples were subjected to separation by high-performance liquid chromatography (HPLC) prior to uv-diode-array detection at X=306 nm. A Phenomenex (Torrance, Calif., USA) Luna 3 micrometer C18 (100×2.00 mm) column was used at 60° C. As mobile phase a gradient of acetonitrile and milliq water (both containing 50 ppm trifluoroacetic acid) was used at a flow of 0.4 ml/min. The gradient profile was linear from 15% acetonitrile to 100% acetonitrile over 20 min. The elution time was approximately and 5.0-5.2 minutes for resveratrol and 8.8-8.9 minutes for trans-pinosylvin.

[0096] The total concentration of stilbenoid intermediates that was produced was then calculated by multiplying the concentrations in the upper phase with a factor of 0.3 and then adding them to the concentrations that were observed in the lower phase. For the PALCPR-control culture, two aliquots of 10 ml of cell broth were collected, and one aliquot was vigorously mixed with 10 ml of 100% of ethanol. The solubility of stilbenoids in ethanol is far higher than in water and thus ensures the determination of levels of stilbenoids that would normally exceed the aquous solubility. Furthermore, stilbenoids that possibly would be bound to the cell-membranes

would be recovered as well. Thus this ethanol-washed aliquot would represent the total amount of stilbenoids produced in PALCPR-control and can be compared to the total amount of stilbenoids produced in the PALCPR-solvent culture. Indeed, a similar ethanol wash performed on the solvent phase did not result in an increase in the titers of stilbenoid- and stilbenoid intermediates, indicating that the solvent phase truly captured all the polar intermediates present in the medium broth or attached to cell membranes. Both aliquots were directly subjected to centrifugation at 3500 g, and the supernatant was analyzed for their content of stilbenoids and their intermediates.

The results are shown in the following table:

	Coumaric acid (mg/l; % total)	Resveratrol (mg/l; % total)	Cinnamic acid (mg/l; % total)	Pinosylvin (mg/l; % total)
PALCPR-solvent				
Upper phase (0.3 L)	—	24.23; 36.2	20.72; 56.9	55.84; 95.2
Lower phase (1 L)	—	12.79; 63.8	4.72; 43.1	0.85; 4.8
Total produced in 1 L	—	20.09; 100	10.93; 100	17.6; 100
PALCPR-control				
Supernatant, ethanol	—	20.01	9.70	22.63
Supernatant, no ethanol	—	11.43	3.59	1.74

[0097] The results demonstrated that the PALCPR strain was able to grow in the presence of a solvent mixture containing 200 ml dodecane, and 100 ml octylacetate, growth rate and biomass yield were Blighty impaired compared to the control fermentation without solvents, however, production of stilbenoids and intermediates was not substantially affected and total titers were similar to the control culture. The solvent mixture was able to capture 95% of the stilbenoid pinosylvin and 36% of the more polar stilbenoid resveratrol. Obviously, the non-polar dodecane fraction in the solvent mixture sufficiently obscured the toxic effects on cells of the more polar and hence more toxic octylaceate fraction. The polarity of the mixture was indeed sufficiently high to capture almost all of the stilbenoid pinosylvin, but did not fully capture the more polar stilbenoid resveratrol.

[0098] The PALCPR control culture produced a persistent brown precipitate that settled on the inner vessel wall and baffles, and moreover fouled vital fermentor parts such as the stirrer shaft, gas-outlet, pH- and dO probe that would endanger a proper control of the fermentation process parts sstirrer shaf precipitates on said fermentor components. The PALCPR-solvent culture, however, did not show fouling of said fermentor components. Instead, a creamy/gelly substance was formed that remained in the fermentation broth, but manifested as an interface between the medium and the solvent phase after centrifugation. This "third" phase did not contain substantial amounts of stilbenoids or intermediates and could be discarded relatively easily.

Example 4

Determination of Intracellular and Extracellular Levels of Stilbenoids in a Batch Culture of an Evolved Strain of PALCPR

[0099] The solvent mixture described in the previous example was not capable to capture all resveratrol produced, therefore a fermentation was initiated to adapt the cells to grow in the presence of a solvent mixture that contained solely the more polar and thus more toxic solvent octylacetate. The cells of the PALCPR-solvent culture described in the previous example were taken as starting point because the presence of the solvent mixture would possibly have already evoked adaptation of cells to solvents. For that, cells of the culture described in the previous example were harvested during mid-exponential-phase and stored at −80° C. in 15% glycerol and subsequently a fermentor, containing the same medium as described in the previous example with 10 g/l glucose and 100 g/l galactose, was then inoculated with cells of said PALCPR-solvent culture. The cells were allowed to fully consume the glucose at a stirring speed of 1000 rpm with an average dissolved oxygen content of above 70% of saturated air. At the onset of galactose consumption, the stirring rate was turned down to 300 rpm, after which slowly 100 ml of octylacetate was added.

[0100] Upon addition of octylacetate consumption of galactose was immediately arrested, the dissolved oxygen content increased to almost 99% of saturated air, O_2— consumption and CO_2 production halted, all indicating that proliferation of cells arrested. However after approximately 170 hours, the CO_2 signal slowly increased, Dissolved oxygen slowly decreased, indicating that cells started to grow. Galactose consumption indeed commenced again. A small arrest in CO_2 production again occurred after 20 hours which, however, recovered after 5 hours, after which the cells grew exponentially with concomitant production of CO_2, consumption of galactose within less than 40 hours. Based upon the CO_2 production the specific growth rate was estimated to be 0.062 1/h (FIG. **2**). The final biomass concentration was 21.6 g/l.

[0101] For the determination of stilbenoids, an aliquot of 25 ml of cell broth was collected, and phase separation was initiated by centrifugation at 3500 g for 5 minutes. Both the upper octylacetate phase and the lower aquous medium phase were collected separately with a pipet and directly analyzed for their content of stilbenoids and intermediates by HPLC. The total concentration of stilbenoid intermediates that was produced was then calculated by dividing the concentrations in the upper phase with a factor of 10 and then adding them to the concentrations that were observed in the lower phase. The results are shown in the following table:

Evolved PALCPR in 2-phase fermentation	Coumaric acid (mg/l; % total)	Resveratrol (mg/l; % total)	Cinnamic acid (mg/l; % total)	Pinosylvin (mg/l; % total)
Upper phase (0.1 L)	233.4; 18.0	163.0; 92.6	408.0; 52.5	32.0; 100
Lower phase (1 L)	106.3; 82.0	1.3; 7.4	36.9; 47.5	0; 0
Total produced in 1 L	129.64; 100	17.6; 100	77.7; 100	3.2; 100

[0102] The results demonstrated clearly that strain PALCPR was able to adapt after 170 hours to the presence of 100 ml octylacetate. Though the growth rate was indeed lower than the PALCPR-control- and PALCPR-solvent culture in the previous example, the final biomass concentration was similar to said cultures.

[0103] The solvent mixture was now able to capture 100% of the stilbenoid pinosylvin and 92% of the more polar stilbenoid resveratrol. That result demonstrated that the polarity of the mixture was indeed sufficiently high to capture both the stilbenoids pinosylvin and resveratrol almost fully to completion. The total amount of resveratrol produced was comparable with the PALCPR-control and PALCPR-solvent cultures in the previous example, however, the culture now produced relatively high amounts of the intermediate coumaric acid, substantial amounts of cinnamic acid and very minor amounts of the stilbenoid pinosylvin. Possibly the observed change in product profile could be the result of the solvent that drained the intermediates away from the cells, thereby changing the intracellular concentrations in the cells and with that influence the product pattern through the kinetic properties of the enzymes. In case that a resveratrol producing strain is preferred, the addition of a solvent clearly could simplify down-stream processing by capturing the resveratrol, and at the same time impairing production of other similar stilbenoids.

[0104] Moreover, similar to the PALCPR-solvent culture in previous example, no persistent brown precipitate was produced that would otherwise have fouled vital fermentor parts, but a creamy/gelly substance was formed that manifested as a "third" phase and which could easily be discarded.

Example 5

Determination of Intracellular and Extracellular Levels of Stilbenoids in a Batch Culture of a Further Evolved Strain of PALCPR

[0105] The evolved PALCPR-evolved-I strain described in the previous example still displayed a long lag-phase to adapt to the solvent phase. In order to further improve said strain in terms of length of lag-phase and growth rate, a 25 ml aliquot of the exponentional growing PALCPR-evolved-I strain was used to inoculate a fermentor, containing a similar medium as described in the previous example. However, the medium now only contained 100 g/l galactose and did contain 100 ml of octylacetate from the beginning of the fermentation. In addition, the stirrer speed was instantly set at 800 rpm. Upon inoculation of the fermentor, consumption of galactose only commenced after approximately 40 hours, indicated by a slow increase in CO_2 signal, a decrease in dissolved oxygen and addition of base. Indeed the cells continued to grow exponentially with concomitant production of CO_2, and consumed the galactose within less than 90 hours. Based upon the CO_2 production the specific growth rate was estimated to be 0.041 1/h (FIG. **3**). The final biomass concentration was 21.5 g/l. The strain is referred to as PALCPR-evolved-II.

[0106] For the determination of stilbenoids, an aliquot of 25 ml of cell broth was collected, and phase separation was initiated by centrifugation at 3500 g for 5 minutes. Both the upper octylacetate phase and the lower aquous medium phase were collected separately with a pipet and directly analyzed for their content of stilbenoids and intermediates by HPLC. The total concentration of stilbenoid intermediates that was produced was then calculated by multiplying the concentrations in the upper phase with a factor of 0.3 and then adding them up to the concentrations that were observed in the lower phase. The results are shown in the following table:

Evolved PALCPR in 2-phase fermentation	Coumaric acid (mg/l; % total)	Resveratrol (mg/l; % total)	Cinnamic acid (mg/l; % total)	Pinosylvin (mg/l; % total)
Upper phase (0.1 L)	383.9; 59.3	210.4; 91.3	163.4; 88.6	30.7; 100
Lower phase (1 L)	26.3; 40.7	2.0; 8.7	2.1; 11.4	0; 0
Total produced in 1 L	64.7; 100	23.0; 100	18.4; 100	3.1; 100

[0107] The results demonstrated clearly that strain PALCPR-evolved-II was able to grow on a medium that contained 100 ml of octyl acetate from the beginning with a stirring speed immediately set at 800 rpm. The lag-phase of 40 hours was already considerably shorter than the 170 hrs described in the previous example, though at the expense of the growth rate that decreased to 0.041 1/h and with that galactose was depleted only after approximately 90 hours. Though the growth rate was indeed lower than the PALCPR-evolved-I culture in the previous example, the final biomass concentration was similar to said culture.

[0108] The solvent mixture was able to capture 100% of the stilbenoid pinosylvin and 91% of the more polar stilbenoid resveratrol. The total amount of resveratrol produced was higher than the PALCPR-evolved-I culture and the PALCPR-control and PALCPR-solvent cultures in the previous examples. However, the culture now produced lower amounts of the intermediates coumaric acid and cinnamic acid, which could be a result of the adaptation of the cells to the solvent.

[0109] Moreover, no persistent brown precipitate was produced that would otherwise have fouled vital fermentor parts,

but a creamy/gelly substance was formed that manifested as a "third" phase and which could easily be discarded.

Example 6

Determination of Intracellular and Extracellular Levels of Stilbenoids in a Batch Culture of an Even Further Evolved Strain of PALCPR

[0110] The evolved PALCPR-evolved-II strain described in the previous example still displayed a shorter lag-phase than PALCPR-evolved-I but the growth rate was rather low with 0.041 1/h. Therefore, to further improve said strain in terms of both length of lag-phase and growth rate, a 50 ml aliquot of the exponentional growing PALCPR-evolved-II strain was used to inoculate a fermentor, containing a similar medium as described in the previous example (thus containing 100 g/l galactose 100 ml of octylacetate from the beginning of the fermentation). The stirrer speed was instantly set at 800 rpm. Upon inoculation of the fermentor, consumption of galactose already commenced after less then 20 hours, indicated by a slow increase in CO_2 signal, a decrease in dissolved oxygen and addition of base. Indeed the cells further grew exponentially with concomitant production of CO_2, and now consumed the galactose within approximately 50 hours. Based upon the CO_2 production the specific growth rate was estimated to be 0.091 1/h (FIG. **4**). The final biomass concentration was 18.9 g/l. The strain is referred to as PALCPR-evolved-III.

[0111] For the determination of stilbenoids, an aliquot of 25 ml of cell broth was collected, and phase separation was initiated by centrifugation at 3500 g for 5 minutes. Both the upper octylacetate phase and the lower aquous medium phase were collected separately with a pipet and directly analyzed for their content of stilbenoids and intermediates by HPLC. The total concentration of stilbenoid intermediates that was produced was then calculated by dividing the concentrations in the upper phase with a factor of 10 and then adding them to the concentrations that were observed in the lower phase. The results are shown in the following table:

Evolved PALCPR in 2-phase fermentation	Coumaric acid (mg/l; % total)	Resveratrol (mg/l; % total)	Cinnamic acid (mg/l; % total)	Pinosylvin (mg/l; % total)
Upper phase (0.1 L)	105.8; 40.0	223.6; 90.3	298.7; 71.3	167.9; 90.3
Lower phase (1 L)	15.9; 60.0	2.4; 9.7	12.0; 28.7	1.8; 9.7
Total produced in 1 L	26.5; 100	24.8; 100	41.9; 100	18.6; 100

[0112] The results demonstrated clearly that strain PALCPR-evolved-III was able to grow on a medium that contained 100 ml of octyl acetate from the beginning with the stirring speed immediately set at 800 rpm. The lag-phase of 20 hours was shortened further compared to the 40 hours lag-phase described in the previous example, and this time the growth rate increased from 0.041 1/h to 0.091 1/h. Concommitantly the galactose was depleted only after approximately 90 hours. Though the growth rate was indeed lower than the PALCPR-evolved-I culture in the previous example, the final biomass concentration was similar to said culture.

[0113] The solvent mixture was able to capture 100% of the stilbenoid pinosylvin and 91% of the more polar stilbenoid resveratrol. The total amount of resveratrol produced was similar to the PALCPR-evolved-II culture but higher than the PALCPR-control and PALCPR-solvent cultures in the previous examples. However, compared to the PALCPR-evolved-III culture, the PALCPR-evolved-III culture produced lower amounts of coumaric acid, higher amounts of cinnamic acid, and considerably higher amounts of pinosylvin. The change in the overall product profile of the phenylpropanoid intermediates could be the result of a further adaptation of the cells to the solvent.

[0114] Moreover, no persistent brown precipitate was produced that would otherwise have fouled vital fermentor parts, but a creamy/gelly substance was formed that manifested as a "third" phase and which could easily be discarded.

Example 7

Stilbenoid Production in *Aspergillus nidulans* AR1 *Aspergillus nidulans* AR1 has Deleted the Following Genes Genes argB2, pyrG89, veA

[0115] a) Construction of a filamentous fungal expression vector, with argB (ornithine carbamoyltransferase) marker.

[0116] The gene encoding argB including the homologous promoter and terminator sequence was amplified from *Aspergillus nidulans* AR1 genomic DNA using forward primer 5-CG GAATTC ATA CGC GGT TTT TTG GGG TAG TCA-3 (SEQ ID NO: 11) and the reverse primer 5-CG CCCGGG TAT GCC ACC TAC AGC CAT TGC GAA-3 (SEQ ID NO: 12) with the 5' overhang containing the restriction sites EcoRI and XmaI respectively. The incorporated restriction sites in the PCR product allowed insertion into pUC19 (New England biolabs, Ipswich, Mass.) digested with EcoRI and XmaI giving pUC19-argB.

[0117] The trpC (Indole-3-glycerol phosphate synthase) terminator was amplified from *A. nidulans* genomic DNA using forward primer 5-GC GGATCC ATA GGG CGC TTA CAC AGT ACA CGA-3 (SEQ ID NO: 13) and the reverse primer 5-CGGAGAGGGCGCGCCCGTGGCGGCCGC GGA TCC ACT TAA CGT TAC TGA-3 SEQ ID NO: 14 with the 5' overhang containing the restriction site BamHI and a 27 base pair adaptamer respectively.

[0118] The gpdA (glyceraldehyde-3-phosphate dehydrogenase) promoter was amplified from *A. nidulans* AR1 genomic DNA using forward primer 5-GCGGCCGC-CACGGGCGCGCCCTCTCCG GCG GTA GTG ATG TCT GCT CAA-3 (SEQ ID NO: 15) and the reverse primer 5-CG AAGCTT TAT AAT TCC CTT GTA TCT CTA CAC-3 SEQ ID NO:16 with the 5' overhang containing a 27 base pair adaptamer and the restriction site HindIII respectively.

The fusion PCR product of fragment trpC and gpdA with the incorporated restriction sites allow insertion into pUC19-argB digested with BamHI and HindIII yielding pAT3.

b) Construction of a filamentous fungal expression vector with pyrG (orotidine-5'-monophosphate decarboxylase) marker for expression of C4H (Cinnamate-4-hydroxylase) in *A. nidulans* AR1.

[0119] The gene encoding C4H was reamplified from the yeast plasmid pESC-URA-PAL2-C4H (WO2006089898) using the forward primer 5-CG G CGCG C ATA ATG GAC CTC CTC TTG CTG GAG-3 SEQ ID NO:17 and the reverse primer 5-GG GC GGCC GC TTA TTA ACA GTT CCT TGG TTT CAT AAC G-3 SEQ ID NO: 18 with the 5' overhang containing the restriction sites BssHII and NotI respectively. The incorporated restriction sites in the PCR product allowed insertion into pAT3 digested with BssHII and NotI giving pAT3-C4H. The construct was verified by restriction enzyme cut and sequencing. The argB marker was removed by using the two following restriction enzymes BsiWI and PciI.

[0120] The gene encoding pyrG including the homologous promoter and terminator sequence was reamplified from *Aspergillus fumigatus* genomic DNA using the forward primer 5-CGT GTAC AATA TTA AT TAA CGAGA GCG AT CGC AAT AAC CGT ATT ACC GCC TTT GAG-3 SEQ ID NO: 19 and reverse primer 5-CGA CATG TAT TCC CGG GAA GAT CTC ATG GTC A-3 SEQ ID NO: 20 with the 5' overhang containing the restriction sites BsrGI, PacI, AsiSI in the forward primer and PciI in the reverse primer. The incorporated restriction sites in the PCR product allowed insertion into pAT3 digested with BsiWI and PciI giving pAT3-C4H-pyrG. The construct was verified by restriction enzyme cut and sequencing.

c) Construction of a filamentous fungal expression vector with argB marker for expression of 4CL1 (4-coumarate-CoA ligase) in *A. nidulans* AR1

[0121] The gene encoding 4CL1 was reamplified from the yeast plasmid pESC-TRP-4CL1-VST1 using the forward primer 5-GCGGAGAGGGCGCG ATG GCG CCA CAA GAA CAA GCA-3 SEQ ID NO: 21 and the reverse primer 5-TGGATCCGCGGCCGC TCA CAA TCC ATT TGC TAG TTT TGC-3 SEQ ID NO: 22. The 4CL1 gene was inserted into a pAT3 vector digested with BssHII and NotI using the In-fusion™ PCR cloning Technology (Clontech, Mountain View, Calif.) to yield pAT3-4CL1. The construct was verified by restriction enzyme cut and sequencing.

d) Construction of a filamentous fungal expression vector with argB marker for expression of VST1 (resveratrol synthase) in *A. nidulans* AR1

[0122] The gene encoding VST1 was reamplified from the yeast plasmid pESC-TRP-4CL1-VST1 using the forward primer 5-CG G CGCG C ATA ATG GCA TCC GTA GAG GAG TTC-3 SEQ ID NO: 23 and the reverse primer 5-GG GC GGCC GC TTA TCA TTA GTT AGT GAC AGT TGG AA-3 SEQ ID NO: 24 with the 5' overhang containing the restriction sites BssHII and NotI respectively. The incorporated restriction sites in the PCR product allowed insertion into pAT3 digested with BssHII and NotI giving pAT3-VST1. The construct was verified by restriction enzyme cut and sequencing.

e) Expression of the pathway leading to pinosylvin in *A. nidulans* AR1 (The strain has deletions (argB2, pyrG89, veA1)) using C4H, 4CL1 and VST1.

[0123] The transformation of the *A. nidulans* AR1 fungal cell was conducted in accordance with methods known in the art by protoplastation using cell wall lysing enzymes (glucanex, novozymes) Tilburn et al., 1983. Random integration of C4H, 4CL1 and VST1 was conducted in two steps. Plasmid pAT3-4CL1 and pAT3-VST1 were linearized using restriction enzyme BmrI and integrated in the genome by co-transformation according to Guerra et al., 2006 utilizing the auxotrophic marker argB. A transformant containing a 4CL1 and VST1 expression cassette was isolated and a successive transformation with pAT3-C4H-pyrG, which was linearized with BmrI, gave a recombinant *A. nidulans* strain containing C4H, 4CL1 and VST1.

Example 8

Stilbenoid Production in *Escherichia coli*

[0124] a) Construction of a bacterial vector for expression of PAL2 in *Escherichia coli.*

[0125] The plasmids that were used in the following examples contained one or more marker genes to allow the microorganism that harbour them to be selected from those which do not. The selection system is based upon dominant markers, e.g. resistance against ampicillin and kanamycin. In addition, the plasmids contained promoter- and terminator sequences that allowed the expression of the recombinant genes. Furthermore, the plasmids contained suitable unique restriction sites to facilitate the cloning of DNA fragments and subsequent identification of recombinants. In this example the plasmids contained either the ampicillin resistance gene, designated as pET16b (Novagen), or the kanamycin resistance gene, designated as pET26b (Novagen).

[0126] The gene encoding PAL2, isolated as described previously, was reamplified by PCR from the plasmid pESC-URA-PAL2 using forward- and reverse primers, with 5' overhangs containing suitable restriction sites. The introduction of said restriction sites at the 5' and 3' ends of the gene allowed ligation of the restricted PCR product into a digested pET16B vector that contained the T7 promoter. The resulting plasmid, pET16B-PAL2, contained the gene encoding PAL2 under the control of the T7 promoter.

b) Construction of a bacterial vector for expression of 4CL1 and VST1 in *Escherichia coli.*

[0127] The gene encoding 4CL1, isolated as described previously, was reamplified by PCR from the plasmid pESC-URA-4CL1-VST1, using forward- and reverse primers, with 5' overhangs containing suitable restriction sites. The introduction of said restriction sites at the 5' and 3' ends of the gene allowed ligation of the restricted PCR product into a digested pET26B vector. The resulting plasmid, pET26B-4CL1, contained the gene encoding for 4CL1 under the control of the T7 promoter from *Lactobacillus* lactis.

[0128] The gene encoding VST1, isolated as described in previously, was reamplified by PCR from the plasmid pESC-URA-4CL1-VST1 using forward- and reverse primers, with 5' overhangs containing suitable restriction sites. The introduction of said restriction sites at the 5' and 3' ends of the gene allowed ligation of the restricted PCR product into a digested pET16B vector. The resulting plasmid, pET16B-VST1, contained the gene encoding VST1 under the control of the T7 promoter. The T7 promoter and the gene encoding VST1 were reamplified as one fragment by PCR from the plasmid pET16B-VST1 using forward and reverse primers, with 5' overhangs containing suitable restriction sites.

[0129] The introduction of said restriction sites at the 5' and 3' ends of the DNA fragment allowed ligation of the restricted PCR product into the digested plasmid pET26B-4CL1. The resulting plasmid, pET26B-4CL1-VST1, contained the genes encoding 4CL1 and VST1, each under the control of their individual T7 promoter. The sequence of the genes encoding 4CL1 and VST1 was verified by sequencing of two different clones of pET26B-4CL1-VST1.

c) Expression of the pathway to pinosylvin in *Escherichia coli*

[0130] *Escherichia coli* strains were transformed with the vectors described in (a) and (b), separately or in combination. The transformation of the bacterial cell was conducted in accordance with methods known in the art, for instance, by using competent cells or by electroporation (see, e.g., Sambrook et al., 1989). Transformants were selected on medium containing the antibiotics ampicillin and kanamycin and streak purified on the same medium.

[0131] *Escherichia coli* strain BL21 (DE3) was transformed separately with the vector pET16B-PAL2 (a), yielding the strain FSEC-PAL2; and with pET26B-4CL1-VST1 (b), yielding strain FSEC-4CL1VST1. In addition, *Escherichia coli* strain BL21 (DE3) was co-transformed with pET16B-PAL2 (a) and pET26B-4CL1-VST1 (n), and the transformed strain was named FSEC-PAL24CL1VST1.

Example 9

Construction of Strain FS09229, Containing the Phenylpropanoid Pathway with Glucose-Constitutive Promoters

I) Isolation of Genes Encoding PAL C4H, 4CL2 and VST1

[0132] 4-coumarate:CoenzymeA ligase (4CL2) SEQ ID NO: 25 (Hamberger and Hahlbrock 2004; Ehlting et al., 1999;) was isolated via PCR from *A. thaliana* cDNA (BioCat, Heidelberg, Germany) using suitable primers.

[0133] The PAL2 gene encoding *Arabidopsis thaliana* resveratrol phenylalanine ammonia lyase (Cochrane et al., 2004) was synthesized by GenScript Corporation (Piscataway, N.J.). The amino acid sequence was used as template to generate a synthetic gene codon (SEQ ID NO: 26) optimized for expression in *S. cerevisiae*. The synthetic PAL2 gene was delivered inserted in *E. coli* pUC57 vector. The synthetic gene was purified from the pUC57 vector by amplifying it by forward primer 5-CAC TAA AGG GCG GCC GCA TGG ACC AAA TTG AAG CA-3 SEQ ID NO: 27 and reverse primer 5-AAT TAA GAG CTC AGA TCT TTA GCA GAT TGG AAT AGG TG-3 SEQ ID NO: 28 and purified from agarose gel using the QiaQuick Gel Extraction Kit (Qiagen).

[0134] The C4H gene encoding *Arabidopsis thaliana* cinnamate-4-hydroxylase (Hamberger and Hahlbrock 2004; Ehlting et al., 1999) was synthesized by GenScript Corporation (Piscataway, N.J.). The amino acid sequence was used as template to generate a synthetic gene (SEQ ID NO: 29) (S codon optimized for expression in *S. cerevisiae*. The synthetic C4H gene was delivered inserted in *E. coli* pUC57 vector. The synthetic gene was purified from the pUC57 vector by amplifying it by forward primer 5-ATT TCC GAA GAA GAC CTC GAG ATG GAT TTG TTA TTG CTG G-3 SEQ ID NO:30 and reverse primer 5-AGT AGA TGG AGT AGA TGG AGT AGA TGG AGT AGA TGG ACA ATT TCT GGG TTT CAT G-3 SEQ ID NO: 31 and purified from agarose gel using the QiaQuick Gel Extraction Kit (Qiagen).

[0135] The ATR2 gene encoding *Arabidopsis thaliana* P450 reductase was synthesized by GenScript Corporation (Piscataway, N.J.). The amino acid sequence was used as template to generate a synthetic gene (SEQ ID NO: 32) codon optimized for expression in *S. cerevisiae*. The synthetic C4H gene was delivered inserted in *E. coli* pUC57 vector. The synthetic gene was purified from the pUC57 vector by amplifying it by forward primer 5-CCA TCT ACT CCA TCT ACT CCA TCT ACT CCA TCT ACT AGG AGG AGC GGT TCG G-3 SEQ ID NO:33 and reverse primer 5-ATC TTA GCT AGC CGC GGT ACC TTA CCA TAC ATC TCT CAG ATA TC-3 SEQ ID NO:34 and purified from agarose gel using the QiaQuick Gel Extraction Kit (Qiagen).

[0136] The VST1 gene encoding *Vitis vinifera* (grapevine) resveratrol synthase (Hain et al., 1993) was synthesized by GenScript Corporation (Piscataway, N.J.). The amino acid sequence was used as template to generate a synthetic gene codon optimized for expression in *S. cerevisiae*. The synthetic VST1 gene (SEQ ID NO: 35) was delivered inserted in *E. coli* pUC57 vector flanked by BamH1 and Xho1 restriction sites. The synthetic gene was amplified using forward primer 5-CCG GAT CCT CAT GGC ATC CGT CGA AGA GTT CAG G-3 SEQ ID NO: 36 and reverse primer 5-CGC TCG AGT TTT AGT TAG TAA CTG TGG GAA CGC TAT GC-3 SEQ ID NO:37 and purified from agarose gel using the QiaQuick Gel Extraction Kit (Qiagen).

II) Construction of a Yeast Vector for Galactose Induced Expression of 4CL2 and VST1

[0137] The gene encoding 4CL2 was isolated as described in section I. The amplified 4CL2 PCR-product using forward primer 5-GCG AAT TCT TAT GAC GAC ACA AGA TGT GAT AGT CAA TGA T-3 SEQ ID NO:38 and reverse primer 5-GCA CTA GTA TCC TAG TTC ATT AAT CCA TTT GCT AGT CTT GC-3 SEQ ID NO:39 was digested with EcoR1/Spe1 and ligated into EcoR1/Spe1 digested pESC-HIS vector (Stratagene), resulting in vector pESC-HIS-4CL2.

[0138] Two different clones of pESC-HIS-4CL2 were sequenced to verify the sequence of the cloned gene.

[0139] The gene encoding VST1 was isolated as described in section I. The amplified synthetic VST1 gene was digested with BamH1/Xho1 and ligated into BamH1/Xho1 digested pESC-HIS-4CL2. The resulting plasmid, pESC-HIS-4CL2-VST1, contained the genes encoding 4CL2 and VST1 under the control of the divergent galactose induced <=GAL1/GAL10=> promoters. The sequence of the gene encoding VST1 was verified by sequencing of two different clones of pESC-HIS-4CL2-VST1 (SEQ ID NO: 40).

III) Construction of a Yeast Vector for Galactose Induced Expression of PAL2 and C4H:ATR2 Fusion Gene

[0140] The gene encoding PAL2 was isolated as described in section I. The amplified PAL2 PCR-product was inserted into NotI/BglII digested pESC-URA vector Stratagene), resulting in vector pESC-URA-PAL2. Two different clones of pESC-URA-PAL2 were sequenced to verify the sequence of the cloned gene.

[0141] The genes encoding C4H and ATR2 were isolated as described in section I. C4H was amplified using forward primer 5-ATT TCC GAA GAA GAC CTC GAG ATG GAT TTG TTA TTG CTG G-3 SEQ ID NO:41 and reverse primer 5-AGT AGA TGG AGT AGA TGG AGT AGA TGG AGT AGA TGG ACA ATT TCT GGG TTT CAT G-3 SEQ ID NO:42. ATR2 was amplified using forward primer 5-CCA TCT ACT CCA TCT ACT CCA TCT ACT CCA TCT ACT AGG AGG AGC GGT TCG G-3 SEQ ID NO:43 and reverse primer 5-ATC TTA GCT AGC CGC GGT ACC TTA CCA TAC ATC TCT CAG ATA TC-3 SEQ ID NO:44.

[0142] The amplified PCR products C4H and ATR2 were used as templates for the creation of the fusion gene C4H:ATR2 using the forward primer 5-ATT TCC GAA GAA GAC CTC GAG ATG GAT TTG TTA TTG CTG G-3 SEQ ID

NO:41 and the reverse primer 5-ATC TTA GCT AGC CGC GGT ACC TTA CCA TAC ATC TCT CAG ATA TC-3 SEQ ID NO:44.

[0143] The Fusion gene C4H:ATR2 gene was inserted into XhoI/KpnI digested pESC-URA-PAL2 by Infusion™ technology (stratagene, La jolla, USA). The resulting plasmid, pESC-URA-PAL2-C4H:ATR2, contained the genes encoding PAL2 and C4H:ATR2 under the control of the divergent galactose induced <=GAL1/GAL10=> promoters. The sequence of the gene encoding C4H:ATR2 was verified by sequencing of two different clones of pESC-URA-PAL2-C4H:ATR2(SEQ ID NO: 45).

IV) Construction of Strong Constitutive Promoter Fragment TDH3

[0144] The 600 base pair TDH3 (GPD) promoter was amplified from *S. cerevisiae* genomic DNA using the forward primer 5'GC GAGCTC AGT TTA TCA TTA TCA ATA CTC GCC ATT TCA AAG SEQ ID NO: 46 containing a Sad restriction site and the reverse primer 5'-CG TCTAGA ATC CGT CGA AAC TAA GTT CTG GTG TTT TAA AAC TAA AA SEQ ID NO:47 containing a Xba1 restriction site. The amplified TDH3 fragment was digested with Sac1/Xba1 and ligated into Sac1/Xba1 digested plasmid pRS416 (Sikorski and Hieter, 1989) as described previously (Mumberg et al, 1995) resulting in plasmid pRS416-TDH3.

V) Construction of Constitutive Strong Promoter Fragment TEF1

[0145] The 400 base pair TEF1 promoter was amplified from *S. cerevisiae* genomic DNA using the forward primer 5'-GC GAGCTC ATA GCT TCA AAA TGT TTC TAC TCC TTT TTT ACT CTT SEQ ID NO:48 containing a Sad restriction site and the reverse primer 5'-CG TCTAGA AAA CTT AGA TTA GAT TGC TAT GCT TTC TTT CTA ATG A SEQ ID NO:49 containing a Xba1 restriction site. The amplified TEF1 fragment was digested with Sac1/Xba1 and ligated into Sac1/Xba1 digested plasmid pRS416 (Sikorski and Hieter, 1989) as described previously (Mumberg et al, 1995) resulting in plasmid pRS416-TEF1.

VI) Construction of Fused Divergent Constitutive TEF1 and TDH3 Promoter Fragment

[0146] A divergent fusion fragment (FIG. **1**) between TEF1 promoter and TDH3 promoter was constructed starting from PRS416-TEF1 and PRS416-TDH3.

[0147] The 600 base pair TDH3 fragment was reamplified from PRS416-TDH3 using the forward primer 5' TTGCGTATTGGGCGCTCTTCC GAG CTC AGT TTA TCA TTA TCA ATA CTC GC SEQ ID NO: 50 containing the underlined overhang for fusion PCR to TEF1 fragment and the reverse primer 5' AT GGATCC TCT AGA ATC CGT CGA AAC TAA GTT CTG SEQ ID NO: 51 containing the underlined BamH1 restriction site. This resulted in a fragment ready for fusion to the below TEF1 fragment.

[0148] The 400 base pair TEF1 fragment including a 277 base pair spacer upstream of the Sad restriction site was reamplified from PRS416-TEF1 using the forward primer 5' AT GAATTC TCT AGA AAA CTT AGA TTA GAT TGC TAT GCT TTC SEQ ID NO: 52 containing the underlined EcoR1 restriction site and the reverse primer 5' TGATAATGATAAACTGAGCTC GGA AGA GCG CCC AAT ACG CAA AC SEQ ID NO: 53 containing the underlined overhang for fusion to the TDH3 fragment. This resulted in a 680 base pair fragment ready for fusion to the TDH3 fragment.

[0149] The 600 base pair TEF1 fragment and the 600 base pair TDH3 fragments were joined together (fused) using fusion PCR with the forward primer 5' AT GAATTC TCT AGA AAA CTT AGA TTA GAT TGC TAT GCT TTC SEQ ID NO: 54 and the reverse primer 5' AT GGATCC TCT AGA ATC CGT CGA AAC TAA GTT CTG SEQ ID NO: 55, resulting in the divergent fragment <=TEF1/TDH3=> (SEQ ID NO: 56).

VII) Construction of a Yeast Vector for Constitutive Expression Induced of 4CL2 and VST1 pesc-HIS-TDH3-4CL2-TEF-VST1

[0150] The vector pESC-HIS-4CL2-VST1 (FIG. **2**) with divergent galactose inducible promoters GAL1/GAL10 was sequentially digested with EcoR1 and BamH1 to remove the GAL1/GAL10 promoters.

[0151] The divergent constitutive <=TEF1/TDH3=> promoter fragment (Sequence ID 56) was reamplified with forward primers 5' ATGAATTC TCT AGA ATC CGT CGA AAC TAA GTT CTG SEQ ID NO: 57 and reverse primers AT GGA TCC TCT AGA AAA CTT AGA TTA GAT TGC TAT GCT TTC TTT CTA A SEQ ID NO: 58 to reverse the orientation of TEF and TDH3 promoters in the final construct, that is to revert construct pESC-HIS-TEF1-4CL2-TDH3-VST1 into pESC-HIS-TDH3-4CL2-TEF1-VST1. The reamplified fragment was sequentially digested with EcoR1 and BamH1 and ligated into the above vector without the GAL1/Gal10 fragment. This resulted in a vector pesc-HIS-TDH3-4CL2-TEF1-VST1 (FIG. **3**) with replaced promoters, from GAL1/Gal10 to TDH3/TEF1 (SEQ ID NO: 59).

VIII) Marker Exchange of the Expression Vector pesc-HIS-TDH3-4CL2-TEF-VST1

[0152] The vector pesc-HIS-TDH3-4CL2-TEF-VST1 with divergent constitutive TDH3/TEF1 promoters was used as template for amplification by PCR with forward primer 5-TCG ACG GAT CTA TGC GGT GTG AAA TAC C-3 (SEQ ID NO: 60) and reverse primer 5-ACT CTC AGT ACA ATC TGC TCT GAT GCC G-3 (SEQ ID NO: 61) removing the His3 expression cassette.

[0153] The Ura3 expression cassette was amplified by PCR using forward primer 5-AGA GCAGATTGTA CTGAGAGT CAT CAG AGC AGA TTG TAC TGA GAG TGC-3 (SEQ ID NO: 62) and reverse primer 5-CAC ACC GCA TAG ATC CGT CGA GGA TTT TGC CGA TTT CGG CCT ATT GG-3 (SEQ ID NO: 63) and template pESC-URA-PAL2-C4H:ATR2. The two PCR fragments were fused by Infusion™ technology (stratagene, La Jolla, USA). This resulted in vector pesc-URA-TDH3-4CL2-TEF-VST1 with replaced auxotrophic marker, from his3 to ura3 (SEQ ID NO: 64).

IX) Construction of a Yeast Vector for Constitutive Expression of PAL2 and C4H:ATR2 Fusion Gene

[0154] The vector pESC-URA-PAL2-C4H:ATR2 with divergent galactose inducible promoters GAL1/GAL10 was sequentially digested with NotI and XhoI to remove the GAL1/GAL10 promoters.

[0155] The divergent constitutive <=TEF1/TDH3=> promoter fragment was re-amplified with forward primer 5-TTC CAG CAA TAA CAA ATC CAT TTT GTA TCT AGA AAA CTT AGA TTA GAT TG-3 SEQ ID NO: 65 and reverse primer 5-CAT TGC TTC AAT TTG GTC CAT TTT GTA TCT AGA ATC CGT CGA AAC TAA GT-3 SEQ ID NO: 66.

The PCR product was sequentially inserted into the above vector without the GAL1/Gal10 fragment using Infusion™ technology (stratagene, La Jolla, USA). This resulted in a vector pESC-URA-TDH3-PAL2-TEF1-C4H:ATR2 with replaced promoters, from GAL1/Gal10 to TEF1/TDH3 (SEQ ID NO: 67).

X) Marker Exchange of the Expression Vector pESC-URA-TDH3-PAL2-TEF1-C4H:ATR2

The vector pESC-URA-TDH3-PAL2-TEF1-C4H:ATR2 with divergent constitutive TDH3/TEF1 promoters was used as template for amplification by PCR with forward primer 5-TGA AAT ACC GCA CAG ATG-3 (SEQ ID NO: 68) and reverse primer 5-CTC TCA GTA CAA TCT GCT-3 (SEQ ID NO: 69) removing the Ura3 expression cassette.

[0156] The His3 expression cassette was amplified by PCR using forward primer 5-AGC AGA TTG TAC TGA GAG GAG CTT GGT GAG CGC TAG GA-3 (SEQ ID NO: 70) and reverse primer 5-C ATC TGT GCG GTA TTT CAC GGT ATT TTC TCC TTA CGC ATC-3 (SEQ ID NO: 71) and template pESC-HIS-4CL2-VST1. The two PCR fragments were fused by Infusion™ technology (stratagene, La Jolla, USA). This resulted in vector pESC-HIS-TDH3-PAL2-TEF1-C4H:ATR2 with replaced auxotrophic marker, from his3 to ura3 (SEQ ID NO: 72).

X1) Expression of the Pathway to Resveratrol in the Yeast *S. cerevisiae* Using PAL2, C4H:ATR2, 4CL2 and VST1

[0157] Yeast strains FS01529 containing the appropriate genetic markers were transformed with the vectors described in sections VIII and X giving FS09229. The transformation of the yeast cell was conducted in accordance with methods known in the art by using competent cells, an alternative being for instance, electroporation (see, e.g., Sambrook et al., 1989). Transformants were selected on medium lacking uracil and histidine and streak purified on the same medium.

Example 10

Determination of Intracellular and Extracellular Levels of Stilbenoids in a Batch Culture of a Further Evolved Strain of PALCPR

[0158] The evolved PALCPR-evolved-III strain described in the previous example was grown in the presence of nonyl-acetate, a similar solvent as octylacetate with one additional carbon atom. A 1 ml aliquot of a glycerol stock that was made from an exponentional growing PALCPR-evolved-III strain was used to inoculate a fermentor, containing a similar medium as described in the previous example, containing only 100 g/l galactose and 100 ml of nonyl-acetate from the beginning of the fermentation. The stirrer speed was instantly set at 800 rpm. Upon inoculation of the fermentor, consumption of galactose only commenced after approximately 70 hours, indicated by a slow increase in CO_2 signal, a decrease in dissolved oxygen and addition of base. Indeed the cells continued to grow exponentially with concomitant production of CO_2, and consumed the galactose within less than 40 hours. Based upon the CO_2 production the specific growth rate was estimated to be 0.098 1/h (FIG. **9**). The final biomass concentration was 36.7 g/l. The cultivation will be referred to as PALCPR-solvent. As a control-experiment said strain was grown in the similar medium without the addition of nonylacetate. Upon inoculation of the fermentor, consumption of galactose commenced already after approximately 40 hours, indicated by an increase in CO_2 signal, a decrease in dissolved oxygen and addition of base. Indeed the cells continued to grow exponentially with concomitant production of CO_2, and consumed the galactose within less than 30 hours. Based upon the CO_2 production the specific growth rate was estimated to be 0.17 1/h. The final biomass concentration was 25.4 g/l. The cultivation will be referred to as PALCPR-control.

[0159] For the determination of stilbenoids, an aliquot of 25 ml of cell broth was collected, and phase separation was initiated by centrifugation at 3500 g for 5 minutes. Both the upper nonylacetate phase and the lower aqueous medium phase were collected separately with a pipette and directly analyzed for their content of stilbenoids and intermediates by HPLC. The total concentration of stilbenoid intermediates that was produced was then calculated by dividing the concentrations in the upper phase with a factor of 10 and then adding them to the concentrations that were observed in the lower phase. The results are shown in the following table:

	Coumaric acid (mg/l; % total)	Resveratrol (mg/l; % total)	Cinnamic acid (mg/l; % total)	Pinosylvin (mg/l; % total)
PALCPR-solvent				
Upper phase (0.1 L)	—	8.27; 100	252.82; 29.6	—
Lower phase (1 L)	—	0.0; 0.0	60.05; 70.4	—
Total produced in 1 L	—	0.83; 100	85.33; 100	—
PALCPR-control				
Supernatant	—	0.48	76.36	—

[0160] The results demonstrated that strain PALCPR-evolved-III was able to grow on a medium that contained 100 ml of nonyl acetate from the beginning with a stirring speed immediately set at 800 rpm. The lag-phase of 70 hours was already considerably longer than the lag-phase of the PALCPR-control fermentation and also a bit longer then a similar fermentation in the presence of octylacetate described in a previous example. The growth rate of 0.098 l/h was similar to the cultivation in the presence of octylacetate and considerably lower than the PALCPR-control fermentation. Surprisingly the biomass concentration was higher than both PALCPR-control and the octylacetate fermentation.

[0161] In both the PALCPR-solvent and PALCPR-control fermentation, only cinnamic acid and resveratrol was produced. The stilbenoid profiles differ therewith previous described control- and solvent fermentations, which could be due to the difference between inocculating with either a fresh

culture (previous examples) or with a glycerol stock (this example). Nevertheless, the solvent mixture was able to capture 100% of the stilbenoid resveratrol whereas only 25% of cinnamic acid was captured. The total amount of resveratrol produced was slightly higher than the PALCPR-control in this example Moreover, the culture produced none of the intermediates coumaric acid and pinosylvin, which could be a result of a further adaptation of the cells to the solvent.

analyzed for their content of stilbenoids and intermediates by HPLC. The total concentration of stilbenoid intermediates that was produced was then calculated by dividing the concentrations in the upper phase with a factor of 10 and then adding them to the concentrations that were observed in the lower phase. The results are shown in the following table:

	Coumaric acid (mg/l; % total)	Resveratrol (mg/l; % total)	Cinnamic acid (mg/l; % total)	Pinosylvin (mg/l; % total)
FS09229-solvent				
Upper phase (0.1 L)	—	160.39; 95.1	39.29; 42.2	373.24; 100
Lower phase (1 L)	—	0.83; 4.9	53.83; 57.8	0.0; 0.0
Total produced in 1 L	—	16.87; 100	9.31; 100	37.32
FS09229-control				
Supernatant	—	43.89	57.81	8.5

Example 11

Determination of Intracellular and Extracellular Levels of Stilbenoids in a Batch Culture of a Non-Evolved Strain Containing the Phenylpropanoid Pathway

[0162] The non-evolved FS09229 strain described in previous examples was grown in the presence of nonyl-acetate. A 1 ml aliquot of a glycerol stock that was made from an exponentional growing FS09229 strain was used to inoculate a fermentor, containing a similar medium as described in the previous example, but now containing 100 g/l glucose and 100 ml of nonyl-acetate from the beginning of the fermentation. The stirrer speed was instantly set at 800 rpm. Upon inoculation of the fermentor, a long lag-phase of approximately 70 hrs set-in, during which an apparent adaptation to the solvent took place. Consumption of glucose only then commenced, indicated by a slow increase in CO_2 signal, a decrease in dissolved oxygen and addition of base. Indeed the cells continued to grow exponentially with concomitant production of CO_2, and consumed the glucose within less than 25 hours. Based upon the CO_2 production the specific growth rate was estimated to be 0.176 1/h (FIG. **10**). The final biomass concentration was 15.5 g/l. The cultivation will be referred to as FS09229-solvent. As a control-experiment said strain was grown in the similar medium without the addition of nonyl-acetate. Upon inoculation of the fermentor, consumption of glucose commenced already after approximately 10 hours, indicated by an increase in CO_2 signal, a decrease in dissolved oxygen and addition of base. Indeed the cells continued to grow exponentially with concomitant production of CO_2, and consumed the glucose within less than 20 hours. Based upon the CO_2 production the specific growth rate was estimated to be 0.22 1/h (FIG. **3**). The final biomass concentration was 12.8 g/l. The cultivation will be referred to as FS09229-control.

[0163] For the determination of stilbenoids, an aliquot of 25 ml of cell broth was collected, and phase separation was initiated by centrifugation at 3500 g for 5 minutes. Both the upper nonylacetate phase and the lower aqueous medium phase were collected separately with a pipette and directly

[0164] The results demonstrated that also the non-evolved strain FS09229 was able to grow on a medium that contained 100 ml of nonyl acetate from the beginning with a stirring speed immediately set at 800 rpm. The lag-phase of 70 hours was already considerably longer than the lag-phase of the FS09229-control fermentation, but rather similar to the fermentation with PALCPR-evolved-III strain described in previous examples. The growth rate of 0.176 l/h was only slightly lower then the FS09229-control cultivation, whereas the biomass concentrations were similar.

[0165] In both the FS09229-solvent and FS09229-control fermentation, only cinnamic acid, pinosylvin and resveratrol was produced. The solvent mixture was able to capture 95% of the stilbenoid resveratrol, 100% of the stilbenoid pinosylvin and 42% of cinnamic acid. The total amount of resveratrol produced was, however lower than the FS09229-control culture, whereas the cinnamic acid- and pinosylvin concentrations were higher. Said difference in the profile of phenylpropanoid pathway intermediates could be a result of a-non optimal adaptation of the cells to the solvent.

[0166] This experiment demonstrates clearly, however, the biocompatibility of nonyl acetate solvent, because non-adapted cells of *S. cerevisiae* are already able to grow on a fermentation medium in the presence of said solvent. For an optimal production of stilbenoids, however, the strain likely needs to be further evolved on said solvent.

Example 12

Adaptation of Microorganisms to the Presence of Solvents

[0167] The present example describes a procedure to rapidly improve the resistance of microorganisms towards non-biocompatible toxic solvents a.

[0168] Cells of a microorganism are subjected to a train of batch fermentations in a fermentor containing a suitable defined medium. The working volume is 1 L and the gas flow rate is set at 1.5 l/m, the temperature and pH is controlled at a desired setting suitable to the organism. The first fermentation contains a suitable carbon source, preferably in the range of 50 g/l to 100 g/l, and stirring rate is kept low to prevent

mixing of the medium- and solvent phase, as illustrated in FIG. **5**A in which a fermentor vessel is shown containing the two separated phases (aqueous) and (solvent) agitated by a stirrer below 200 rpm. At the onset of carbon-source consumption, a mixture of solvents is added. The mixture contains 10 ml of solvent (now referred to as "extractant") that is a good extractant for the desired product, but non-biocompatible with the cells, and 40 ml of a biocompatible solvent (now referred to as "biocompatible solvent") that is a poor extractant for the desired product. After depletion of the carbon source the strain is harvested and used for a next fermentation. The next fermentation is performed with said harvested strain using similar fermentation conditions as to the previous one, but now with addition of a mixture of 50 ml extractant and 100 ml biocompatible solvent. In case the cells are able to grow in the presence of said solvent mixture, the strain is harvested after carbon source depletion and used for a next fermentation. The next fermentation is performed with said harvested strain, using similar conditions as to the previous one, but now the stirring rate is increased to 1000 rpm halfway through the consumption of the carbon source, causing mixing of the solvent phase with the medium phase as illustrated in FIG. **5**B. In case the cells remain able to grow when the phases are mixed, the strain is harvested and used for a next fermentation. The next fermentation is then performed with said harvested strain using similar conditions as to the previous one, but now with addition of a mixture of 100 ml extractant and 100 ml biocompatible solvent. In case the increase of the extractant to 100 ml causes the arrest of cell growth, indicated by a halt in carbon source consumption, the amount of biocompatible solvent can be increased to, for instance to 200 ml, in order to further obscure the toxic effect of extractant. If necessary the amount of biocompatible solvent can even be increased further up to the point that cells can grow in the presence of the solvent mixture. In case cells are indeed able to grow in the presence of said solvent mixture at high stirring rate, the strain is harvested and stored in a solution of 15% glycerol at −80° C.

[0169] In case that the polarity of said solvent mixture is too low to capture sufficient amounts of desired polar products, a further train of fermentations can be initiated to adapt the cells to grow in the presence of solely the more polar and thus more toxic solvent extractant. The cells obtained from the previously described adaptation rounds are taken as starting point because the presence of the solvent mixture should possibly have already evoked adaptation of cells to solvents. For that, the glycerol stocks cells of the cultures described in the previous example is used to inoculate a fermentor, containing the same medium as described in the previous example with a suitable carbon source in the range of 50- to 100 g/l. The cells are allowed to fully consume the carbon source at a stirring speed of 1000 rpm with average dissolved oxygen content of above 70% of saturated air. At the onset of carbon source consumption, the stirring rate is turned down to 300 rpm, after which slowly 100 ml of extractant is added.

Upon addition of extractant, consumption of carbon source is arrested, the dissolved oxygen content increases to almost 99% of saturated air, O_2-consumption and CO_2 production halts, all indicating that the proliferation of cells arrested. However after a long lag-phase, the CO_2 signal slowly increases, dissolved oxygen slowly decreases, indicating that cells start to grow, and consumption of carbon source commences again.

[0170] To further improve said strain in terms of reducing the length of the lag-phase and growth rate, a 25 ml aliquot of the exponentional growing strain as described above is used to inoculate a fermentor, containing a similar medium as described previously. However, the medium now contains 100 ml of extractant from the very beginning of the fermentation. In addition, the stirrer speed is instantly set at 800 rpm. Upon inoculation of the fermentor, consumption of the carbon source commences after a shorter lag-phase than before. To further improve said strain in terms of both length of lag-phase and growth rate, another adaption round follows with a 50 ml aliquot of the exponentional growing adapted-strain, in the presence of 100 ml of extractant and with the stirring rate instantly set at 800 rpm. The number of adaptation rounds is repeated until a satisfactory reduction of lag-phase, and sufficiently high growth-rates are reached.

Example 13

Determination of Intracellular and Extracellular Levels of Stilbenoids in Shakeflask Cultures of FS06112, in the Presence of the Solvent Octyl Acetate

[0171] A glycerol stock of strain FS06112 was used to inoculate 500 ml baffled shake flasks that contained 100 ml modified M9 medium consisting of 4.5 g/l glycerol, 1.5 g/l yeast extract, 3 g/l K_2HPO_4, 6.8 g/l Na_2HPO_4, 0.5 g/l NaCl, 1 g/l NH_4C, 50 μg/ml ampicillin and 50 μg/ml kanamycin; the initial pH was set at 7.0. The shakeflasks were incubated at ambient temperature, and were mixed with a magnetic stirring bar at approximately 150 rpm. After 5 hours, isopropyl β-thiogalactopyranoside (IPTG) was added at a final concentration of 4 mM, as an inducer of the T7 promoter that was in front of each of the three genes TAL, 4CL and VST. After one hour an aliquot of 10 ml of the solvent octyl acetate was added to one shakeflask that is now referred to as FS06112-solvent; the shakeflask culture without solvent will be referred to as FS06112-control. After an incubation period of approximately 24 hours, 2.5 g/l glycerol was consumed in both the FS06112-solvent and -control culture and both the medium and solvent phases were analyzed for the presence of resveratrol.

[0172] For the determination of stilbenoids, an aliquot of 50 ml of cell broth was collected, and phase separation was initiated by centrifugation at 4500 g for 10 minutes. Both the upper methyl decanoate phase and the lower aqueous medium phase were collected separately with a pipette and directly analyzed for their content of stilbenoids and intermediates by HPLC. The total concentration of stilbenoid intermediates that was produced was then calculated by dividing the concentrations in the upper phase with a factor of 10 and then adding them to the concentrations that were observed in the lower phase. The results are shown in the following table:

Solvent: octyl acetate	Coumaric acid (mg/l; % total)	Resveratrol (mg/l; % total)	Cinnamic acid (mg/l; % total)	Pinosylvin (mg/l; % total)
FS06112-solvent				
Upper phase (0.1 L)	—	3.03; 100	—	—
Lower phase (1 L)	—	0.0; 0.0	—	—
Total produced in 1 L	—	0.30; 100	—	—
FS06112-control				
Supernatant	—	0.85	—	—

The results demonstrated that strain FS06112 was able to grow in a shakeflask on a medium to which 10 ml of octyl acetate was added at the onset of induction of resveratrol production. Both FS06112-solvent- and FS06112-control cultivation consumed 2.5 g/l of glycerol within 24 hrs.

[0173] In both the FS06112-control and FS06112-solvent cultivation, resveratrol was produced without co-production of any of the other pathway intermediates coumaric acid and pinosylvin. The solvent was able to capture 100% of the stilbenoid resveratrol, but the total amount of resveratrol produced was lower than the FS06112-control cultivation.

Example 14

Construction *E. coli* Strain FS06112, Containing the "TAL"-Phenylpropanoid Pathway with Inducible Promoters

[0174] I) Construction of a Bacterial Vector for Expression of TAL in *Escherichia coli.*

[0175] An ammonia lyase from *Rhodobacter capsulatus*, which was codon-optimized for use in *S. cerevisiae*, was used as the basis for the construction of a bacterial vector for expression of TAL in *Escherichia coli*. The coding sequence of tyrosine ammonia lyase (TAL) from *Rhodobacter capsulatus* (Kyndt et al., 2002; SEQ ID NO: 73 (nucleotide) and 74 (amino acid)) was codon optimized for expression in *S. cerevisiae* using the online service back translation tool at www.entelechon.com, yielding sequence SEQ ID NO: 75, which again expresses SEQ ID NO: 74. Oligos for the synthetic gene assembly were constructed at MWG Biotech and the synthetic gene was assembled by PCR using a slightly modified method protocol of from Martin et al. (2003). The amplified synthetic TAL gene was digested with EcoR1/Spe1 and ligated into EcoR1/Spe1-digested pESC-URA vector. The resulting plasmid, pESC-URA-TAL, contained the gene encoding for TAL under the control of the divergent GAL1/GAL10 promoter. The sequence was verified by sequencing of two different clones of pESC-URA-TAL. The gene encoding tyrosine ammonia lyase (TAL) was reamplified by PCR from the yeast plasmid pESC-URA-TAL described in using the forward primer 5'-CCG CTCGAG CGG ATG ACC CTG CAA TCT CAA ACA GCT AAA G-3' SEQ ID NO: 76 and the reverse primer 5'-GC GGATCC TTA AGC AGG TGG ATC GGC AGC T-3' SEQ ID NO: 77 with 5' overhangs containing the restriction sites XhoI and BamHI, respectively. The introduction of restriction sites at the 5' and 3' ends of the gene allowed ligation of the restricted PCR product into a pET-16b vector (Novagen), digested with XhoI and BamHI to yield pET16b-TAL. The pET-16b vector contained both the gene for ampicillin resistance and the T7 promoter. Hence, above procedure resulted in a vector that contained the gene encoding TAL under the control of the T7 promoter. The sequence of the gene encoding TAL was verified by sequencing of one clone of pET16b-TAL.

II) Construction of a Bacterial Vector for Expression of 4CL and VST in *Escherichia coli.*

[0176] The gene encoding 4-Coumarate-CoA ligase (4CL1) was reamplified by PCR from the yeast plasmid pESC-TRP-4CL1-VST1 using the forward primer 5'-TG CCATGG CA ATGGCGCCAC AAGAACAAGC AGTTT-3' SEQ ID NO: 78 and the reverse primer 5'-GC GGATCC CCT TCA CAA TCC ATT TGC TAG TTT TGCC-3' SEQ ID NO: 79 with 5' overhangs containing the restriction sites NcoI and BamHI, respectively. The introduction of restriction sites at the 5' and 3' ends of the gene allowed ligation of the restricted PCR product into a pET16b vector (Novagen) digested with NcoI and BamHI. The resulting plasmid, pET16b-4CL1, contained the gene encoding for 4CL1 under the control of the T7 promoter. Both the T7 promoter and the gene encoding 4CL1 were reamplified as one fragment by PCR from the plasmid pET16b-4CL1 using the forward primer (A) 5'-GA-CAAGCTTGCGGCC AGA TCT CGA TCC CGC GAA ATT AAT ACG-3' SEQ ID NO: 80 and the reverse primer (B) 5'-TGCTCGAGTGCGGCC TCA CAA TCC ATT TGC TAG TTT TGCC-3' SEQ ID NO: 81. The 4CL1 gene was inserted into a pET26b-VST1 vector (Novagen) digested with Not1 using the In-fusion™ PCR cloning Technology (Clontech, Mountain View, Calif.) to yield pET26b-VST1-4CL1. The resulting plasmid, pET26b-VST1-4CL1, contained the two genes 4CL1 and VST1 that each are under control of an individual T7 promoter. The sequences of the genes, promoters and terminator were verified by sequencing of four clones of pET26b-VST1-4CL1.

[0177] The gene encoding grape resveratrol synthase (VST1) was reamplified by PCR from the yeast plasmid pESC-TRP-4CL1-VST1, using the forward primer 5'-CGC CATATG ATG GCA TCC GTA GAG GAG TTC AGA A-3' SEQ ID NO: 82 and the reverse primer 5'-CC GGATCC TCA TTA GTT AGT GAC AGT TGG AAC AGA GT-3' SEQ ID NO: 83. The VST1 gene was inserted into a pET26b vector (Novagen) digested with Nde1 and BamH1 using the In-fusion™ PCR cloning Technology (Clontech, Mountain View, Calif.) to yield pET26b-VST1. The pET26b vector contained both the gene for kanamycin resistance and the T7 promoter. Hence, above procedure resulted in a vector that contained the gene encoding VST1 under the control of the T7 promoter. Cloning between the Nde1 and Bamh1 restriction sites enabled the removal of N-terminal pET26b pelB secretion signal sequence, which would otherwise enable targeting of the expressed protein to the *E. coli* periplasmic space. An

extra VST1 copy was cloned into a third vector encoding chloramphenicol resistance by reamplifying VST1 gene by PCR from the yeast plasmid pESCTRP-4CL1-VST1, using the forward primer 5'-AAGGAGATATACATATG ATG GCA TCC GTA GAG GAG TTC AGA A-3' SEQ ID NO: 84 and the reverse primer 5'-CTTTACCAGACTC GAG TCA TTA GTT AGT GAC AGT TGG AAC AGA GT-3' SEQ ID NO: 85. The VST1 gene was inserted into a pACYCDuet-1 vector (Novagen) digested with Nde1 and Xho1 using the In-fusion™ PCR cloning Technology (Clontech, Mountain View, Calif.) to yield pACYCDuet-VST1.

III) Expression of the Pathway to Resveratrol in *Escherichia coli*, Using TAL, 4CL and VST.

[0178] The transformation of the bacterial cell was conducted in accordance with methods known in the art, for instance, by using competent cells or by electroporation (see, e.g., Sambrook et al., 1989). The *E. coli* strain BL21 (DE3) (Novagen) was co-transformed with the three vectors pET16b-TAL, pET26b-VST-4CL, and pACYCDuet-VST1. Transformants were selected on Luria-Bertani (LB) medium with 50 mg/l ampicillin, 50 mg/l kanamycin and 50 mg/l chloramphenicol. This resulted in a strain harboring three plasmids pET16b-TAL, pET26b-VST-4CL, and pACYC-Duet-VST1 with the full "TAL"-resveratrol pathway and double VST1 copies (FS06111). It was originally thought, before extraction procedures had been optimized, that an extra copy of VST1 gene was needed on a third plasmid for efficient expression of the resveratrol pathway. However, after optimization of extraction procedures, it was demonstrated that a strain harbouring only the two plasmids pET16-TAL and pET26-4CL1-VST1, performed as well as the three-plasmid strain with the extra VST1 copy (FS06111). The strain expressing the resveratrol pathway with two plasmids (FS06112), therefore, was obtained by restreaking FS06111 on LB agar plates, containing only 50 mg/l ampicillin and 50 mg/l kanamycin, but lacking chloramphenicol; with no selection pressure from chloramphenicol, the third plasmid was eventually lost.

Example 15

Determination of Intracellular and Extracellular Levels of Stilbenoids in Shakeflask Cultures of FS06112, in the Presence of the Solvent Methyl-Decanoate

[0179] A glycerol stock of strain FS06112 was used to inoculate 500 ml baffled shake flasks that contained 100 ml modified M9 medium consisting of 4.5 g/l glycerol, 1.5 g/l yeast extract, 3 g/l K_2HPO_4, 6.8 g/l Na_2HPO_4, 0.5 g/l NaCl, 1 g/l NH_4C, 50 μg/ml ampicillin and 50 μg/ml kanamycin; the initial pH was set at 7.0. The shakeflasks were incubated at ambient temperature, and were mixed with a magnetic stirring bar at approximately 150 rpm. After 5 hours, isopropyl β-thiogalactopyranoside(IPTG) was added at a final concentration of 4 mM, as an inducer of the T7 promoter that was in front of each of the three genes TAL, 4CL and VST. After one hour an aliquot of 10 ml of the solvent methyl decanoate was added to one shakeflask that is now referred to as FS06112-solvent; the shakeflask culture without solvent will be referred to as FS06112-control. After an incubation period of approximately 48 hours, the glycerol was depleted and the medium and solvent phases were analyzed for the presence of resveratrol.

[0180] For the determination of stilbenoids, an aliquot of 50 ml of cell broth was collected, and phase separation was initiated by centrifugation at 4500 g for 10 minutes. Both the upper methyl decanoate phase and the lower aqueous medium phase were collected separately with a pipette and directly analyzed for their content of stilbenoids and intermediates by HPLC. The total concentration of stilbenoid intermediates that was produced was then calculated by dividing the concentrations in the upper phase with a factor of 10 and then adding them to the concentrations that were observed in the lower phase. The results are shown in the following table:

Solvent: methyl decanoate	Coumaric acid (mg/l; % total)	Resveratrol (mg/l; % total)	Cinnamic acid (mg/l; % total)	Pinosylvin (mg/l; % total)
FS06112-solvent				
Upper phase (0.1 L)	—	8.79; 100	—	—
Lower phase (1 L)	—	0.0; 0.0	—	—
Total produced in 1 L	—	0.88; 100	—	—
FS06112-control				
Supernatant	—	1.08	—	—

[0181] The results demonstrated that strain FS06112 was able to grow in a shakeflask on a medium that to which 10 ml of methyl-decanoate was added at the onset of induction of resveratrol production. The FS06112-solvent cultivation consumed the glycerol within 48 hrs, which was only slightly longer than the 30 hours in which the FS06112-control cultivation consumed the glycerol.

[0182] In both the FS06112-control and FS06112-solvent cultivation, resveratrol was produced without co-production of any of the other pathway intermediates coumaric acid and pinosylvin. In addition, in the chromatogram of the upper-solvent phase of the FS06112-solvent cultivation a small peak could be observed with a retention time and UV-spectrum that resembled those of cis-resveratrol. Most likely, a small fraction of the produced trans-resveratrol was converted into cis-resveratrol, which could be an effect of the presence of the solvent. The solvent was able to capture 100% of the stilbenoid resveratrol, and the total amount of resveratrol produced was slightly lower than the FS06112-control cultivation. However, the small peak that allegedly represented cis-resveratrol was approximately 25% of the area of the trans-resveratrol peak, which implied that the total resveratrol content produced in the FS06112-solvent cultivation could be equal or even slightly higher than in the FS06112-control cultivation.

Example 16

Determination of Intracellular and Extracellular Levels of Stilbenoids in Shakeflask Cultures of FS06112, in the Presence of the Solvent Undecanone

[0183] A glycerol stock of strain FS06112 was used to inoculate 500 ml baffled shake flasks that contained 100 ml modified M9 medium consisting of 4.5 g/l glycerol, 1.5 g/l yeast extract, 3 g/l K_2HPO_4, 6.8 g/l Na_2HPO_4, 0.5 g/l NaCl, 1 g/l NH_4C, 50 μg/ml ampicillin and 50 μg/ml kanamycin; the initial pH was set at 7.0. The shakeflasks were incubated at ambient temperature, and were mixed with a magnetic stirring bar at approximately 150 rpm. After 5 hours, isopropyl β-thiogalactopyranoside (IPTG) was added at a final concentration of 4 mM, as an inducer of the T7 promoter that was in front of each of the three genes TAL, 4CL and VST. After one hour an aliquot of 10 ml of the solvent undecanone was added to one shakeflask that is now referred to as FS06112-solvent; the shakeflask culture without solvent will be referred to as FS06112-control. After an incubation period of approximately 48 hours, the glycerol was depleted and the medium and solvent phases were analyzed for the presence of resveratrol.

[0184] For the determination of stilbenoids, an aliquot of 50 ml of cell broth was collected, and phase separation was initiated by centrifugation at 4500 g for 10 minutes. Both the upper undecanone phase and the lower aqueous medium phase were collected separately with a pipette and directly analyzed for their content of stilbenoids and intermediates by HPLC. The total concentration of stilbenoid intermediates that was produced was then calculated by dividing the concentrations in the upper phase with a factor of 10 and then adding them to the concentrations that were observed in the lower phase. The results are shown in the following table:

Solvent: Undecanone	Coumaric acid (mg/l; % total)	Resveratrol (mg/l; % total)	Cinnamic acid (mg/l; % total)	Pinosylvin (mg/l; % total)
FS06112-solvent				
Upper phase (0.1 L)	—	8.82; 100	—	—
Lower phase (1 L)	—	0.0; 0.0	—	—
Total produced in 1 L	—	0.88; 100	—	—
FS06112-control				
Supernatant	—	1.08	—	—

The results demonstrated that strain FS06112 was able to grow in a shakeflask on a medium that to which 10 ml of undecanone was added at the onset of induction of resveratrol production. The FS06112-solvent cultivation consumed the glycerol within 48 hrs, which was only slightly longer than the 30 hours in which the FS06112-control cultivation consumed the glycerol.

[0185] In both the FS06112-control and FS06112-solvent cultivation, resveratrol was produced without co-production of any of the other pathway intermediates coumaric acid and pinosylvin. In addition, the determination of the resveratrol content in the solvent phase was sometimes complicated by a considerable shift in retention time of the resveratrol peak. Most likely the high concentration of undecanone in the sample interfered with the a-polar interactions between the resveratrol molecule and the stationary phase. Apparently undecanone interfered more intensively with the analysis than all the other solvents used in the previous examples because no drastic retention times shifts have been observed with said other solvents. Still it was possible to render a quantitative analysis and to establish that undecanone was able to capture 100% of the stilbenoid resveratrol, and that the total amount of resveratrol produced was slightly lower than the FS06112-control cultivation. However, with the uncertainty of the retention time shift and its effect on the peak shape the total resveratrol content produced in the FS06112-solvent cultivation could be considered at least equal to that of the FS06112-control cultivation.

[0186] In this specification, unless expressly otherwise indicated, the word 'or' is used in the sense of an operator that returns a true value when either or both of the stated conditions is met, as opposed to the operator 'exclusive or' which requires that only one of the conditions is met. The word 'comprising' is used in the sense of 'including' rather than in to mean 'consisting of'. All prior teachings acknowledged above are hereby incorporated by reference. No acknowledgement of any prior published document herein should be taken to be an admission or representation that the teaching thereof was common general knowledge in Australia or elsewhere at the date hereof.

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SEQUENCE LISTING

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cacgctttgt ccaacggtga gactgagaag aatgcagtga cttcgatctt tcaaaagatt   1860

ggagcttttg aagaggagct taaggctgtg cttccaaagg aagttgaagc ggctagagcg   1920

gcttatggga atggaactgc gccgattcct aaccggatta aggaatgtag gtcgtatccg   1980

ttgtataggt tcgtgaggga agagcttgga acgaagttgt tgactggaga aaaggttgtg   2040

tctccgggag aggagtttga taaggtcttc actgctatgt gtgaaggtaa acttattgat   2100

ccgttgatgg attgtctcaa ggaatggaac ggagctccga ttccgatttg ctaa          2154
```

<210> SEQ ID NO 2
<211> LENGTH: 717
<212> TYPE: PRT
<213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 2

```
Met Asp Gln Ile Glu Ala Met Leu Cys Gly Gly Gly Glu Lys Thr Lys
1               5                   10                  15

Val Ala Val Thr Thr Lys Thr Leu Ala Asp Pro Leu Asn Trp Gly Leu
            20                  25                  30

Ala Ala Asp Gln Met Lys Gly Ser His Leu Asp Glu Val Lys Lys Met
        35                  40                  45

Val Glu Glu Tyr Arg Arg Pro Val Val Asn Leu Gly Gly Glu Thr Leu
    50                  55                  60

Thr Ile Gly Gln Val Ala Ala Ile Ser Thr Val Gly Gly Ser Val Lys
65                  70                  75                  80

Val Glu Leu Ala Glu Thr Ser Arg Ala Gly Val Lys Ala Ser Ser Asp
                85                  90                  95

Trp Val Met Glu Ser Met Asn Lys Gly Thr Asp Ser Tyr Gly Val Thr
            100                 105                 110

Thr Gly Phe Gly Ala Thr Ser His Arg Arg Thr Lys Asn Gly Thr Ala
        115                 120                 125

Leu Gln Thr Glu Leu Ile Arg Phe Leu Asn Ala Gly Ile Phe Gly Asn
    130                 135                 140

Thr Lys Glu Thr Cys His Thr Leu Pro Gln Ser Ala Thr Arg Ala Ala
145                 150                 155                 160

Met Leu Val Arg Val Asn Thr Leu Leu Gln Gly Tyr Ser Gly Ile Arg
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                165                 170                 175

Phe Glu Ile Leu Glu Ala Ile Thr Ser Leu Leu Asn His Asn Ile Ser
            180                 185                 190

Pro Ser Leu Pro Leu Arg Gly Thr Ile Thr Ala Ser Gly Asp Leu Val
        195                 200                 205

Pro Leu Ser Tyr Ile Ala Gly Leu Leu Thr Gly Arg Pro Asn Ser Lys
    210                 215                 220

Ala Thr Gly Pro Asp Gly Glu Ser Leu Thr Ala Lys Glu Ala Phe Glu
225                 230                 235                 240

Lys Ala Gly Ile Ser Thr Gly Phe Phe Asp Leu Gln Pro Lys Glu Gly
                245                 250                 255

Leu Ala Leu Val Asn Gly Thr Ala Val Gly Ser Gly Met Ala Ser Met
            260                 265                 270

Val Leu Phe Glu Ala Asn Val Gln Ala Val Leu Ala Glu Val Leu Ser
        275                 280                 285

Ala Ile Phe Ala Glu Val Met Ser Gly Lys Pro Glu Phe Thr Asp His
    290                 295                 300

Leu Thr His Arg Leu Lys His His Pro Gly Gln Ile Glu Ala Ala Ala
305                 310                 315                 320

Ile Met Glu His Ile Leu Asp Gly Ser Ser Tyr Met Lys Leu Ala Gln
                325                 330                 335

Lys Val His Glu Met Asp Pro Leu Gln Lys Pro Lys Gln Asp Arg Tyr
            340                 345                 350

Ala Leu Arg Thr Ser Pro Gln Trp Leu Gly Pro Gln Ile Glu Val Ile
        355                 360                 365

Arg Gln Ala Thr Lys Ser Ile Glu Arg Glu Ile Asn Ser Val Asn Asp
    370                 375                 380

Asn Pro Leu Ile Asp Val Ser Arg Asn Lys Ala Ile His Gly Gly Asn
385                 390                 395                 400

Phe Gln Gly Thr Pro Ile Gly Val Ser Met Asp Asn Thr Arg Leu Ala
                405                 410                 415

Ile Ala Ala Ile Gly Lys Leu Met Phe Ala Gln Phe Ser Glu Leu Val
            420                 425                 430

Asn Asp Phe Tyr Asn Asn Gly Leu Pro Ser Asn Leu Thr Ala Ser Ser
        435                 440                 445

Asn Pro Ser Leu Asp Tyr Gly Phe Lys Gly Ala Glu Ile Ala Met Ala
    450                 455                 460

Ser Tyr Cys Ser Glu Leu Gln Tyr Leu Ala Asn Pro Val Thr Ser His
465                 470                 475                 480

Val Gln Ser Ala Glu Gln His Asn Gln Asp Val Asn Ser Leu Gly Leu
                485                 490                 495

Ile Ser Ser Arg Lys Thr Ser Glu Ala Val Asp Ile Leu Lys Leu Met
            500                 505                 510

Ser Thr Thr Phe Leu Val Gly Ile Cys Gln Ala Val Asp Leu Arg His
        515                 520                 525

Leu Glu Glu Asn Leu Arg Gln Thr Val Lys Asn Thr Val Ser Gln Val
    530                 535                 540

Ala Lys Lys Val Leu Thr Thr Gly Ile Asn Gly Glu Leu His Pro Ser
545                 550                 555                 560

Arg Phe Cys Glu Lys Asp Leu Leu Lys Val Val Asp Arg Glu Gln Val
                565                 570                 575
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Phe Thr Tyr Val Asp Asp Pro Cys Ser Ala Thr Tyr Pro Leu Met Gln
            580                 585                 590

Arg Leu Arg Gln Val Ile Val Asp His Ala Leu Ser Asn Gly Glu Thr
        595                 600                 605

Glu Lys Asn Ala Val Thr Ser Ile Phe Gln Lys Ile Gly Ala Phe Glu
    610                 615                 620

Glu Glu Leu Lys Ala Val Leu Pro Lys Glu Val Glu Ala Ala Arg Ala
625                 630                 635                 640

Ala Tyr Gly Asn Gly Thr Ala Pro Ile Pro Asn Arg Ile Lys Glu Cys
                645                 650                 655

Arg Ser Tyr Pro Leu Tyr Arg Phe Val Arg Glu Glu Leu Gly Thr Lys
            660                 665                 670

Leu Leu Thr Gly Glu Lys Val Val Ser Pro Gly Glu Glu Phe Asp Lys
        675                 680                 685

Val Phe Thr Ala Met Cys Glu Gly Lys Leu Ile Asp Pro Leu Met Asp
    690                 695                 700

Cys Leu Lys Glu Trp Asn Gly Ala Pro Ile Pro Ile Cys
705                 710                 715


<210> SEQ ID NO 3
<211> LENGTH: 1518
<212> TYPE: DNA
<213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 3

atggacctcc tcttgctgga gaagtcttta atcgccgtct tcgtggcggt gattctcgcc     60

acggtgattt caaagctccg cggcaagaaa ttgaagctac ctccaggtcc tataccaatt    120

ccgatcttcg gaaactggct tcaagtcgga gatgatctca accaccgtaa tctcgtcgat    180

tacgctaaga aattcggcga tctcttcctc ctccgtatgg gtcagcgaaa cctagtcgtc    240

gtctcctcac cggatctaac aaaggaagtg ctcctcactc aaggcgttga gtttggatcc    300

agaacgagaa acgtcgtgtt cgacattttc accgggaaag gtcaagatat ggtgttcact    360

gtttacggcg agcattggag gaagatgaga agaatcatga cggttccttt cttcaccaac    420

aaagttgttc aacagaatcg tgaaggttgg gagtttgaag cagctagtgt tgttgaagat    480

gttaagaaga atccagattc tgctacgaaa ggaatcgtgt tgaggaaacg tttgcaattg    540

atgatgtata acaatatgtt ccgtatcatg ttcgatagaa gatttgagag tgaggatgat    600

cctcttttcc ttaggcttaa ggctttgaat ggtgagagaa gtcgattagc tcagagcttt    660

gagtataact atggagattt cattcctatc cttagaccat tcctcagagg ctatttgaag    720

atttgtcaag atgtgaaaga tcgaagaatc gctcttttca agaagtactt tgttgatgag    780

aggaagcaaa ttgcgagttc taagcctaca ggtagtgaag gattgaaatg tgccattgat    840

cacatccttg aagctgagca gaagggagaa atcaacgagg acaatgttct ttacatcgtc    900

gagaacatca atgtcgccgc gattgagaca acattgtggt ctatcgagtg gggaattgca    960

gagctagtga accatcctga aatccagagt aagctaagga acgaactcga cacagttctt   1020

ggaccgggtg tgcaagtcac cgagcctgat cttcacaaac ttccatacct tcaagctgtg   1080

gttaaggaga ctcttcgtct gagaatggcg attcctctcc tcgtgcctca catgaacctc   1140

catgatgcga agctcgctgg ctacgatatc ccagcagaaa gcaaaatcct tgttaatgct   1200
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tggtggctag caaacaaccc caacagctgg aagaagcctg aagagtttag accagagagg 1260

ttctttgaag aagaatcgca cgtggaagct aacggtaatg acttcaggta tgtgccattt 1320

ggtgttggac gtcgaagctg tcccgggatt atattggcat tgcctatttt ggggatcacc 1380

attggtagga tggtccagaa cttcgagctt cttcctcctc caggacagtc taaagtggat 1440

actagtgaga aaggtggaca attcagcttg cacatcctta accactccat aatcgttatg 1500

aaaccaagga actgttaa 1518

<210> SEQ ID NO 4
<211> LENGTH: 505
<212> TYPE: PRT
<213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 4

Met Asp Leu Leu Leu Leu Glu Lys Ser Leu Ile Ala Val Phe Val Ala
1 5 10 15

Val Ile Leu Ala Thr Val Ile Ser Lys Leu Arg Gly Lys Lys Leu Lys
20 25 30

Leu Pro Pro Gly Pro Ile Pro Ile Pro Ile Phe Gly Asn Trp Leu Gln
35 40 45

Val Gly Asp Asp Leu Asn His Arg Asn Leu Val Asp Tyr Ala Lys Lys
50 55 60

Phe Gly Asp Leu Phe Leu Leu Arg Met Gly Gln Arg Asn Leu Val Val
65 70 75 80

Val Ser Ser Pro Asp Leu Thr Lys Glu Val Leu Leu Thr Gln Gly Val
85 90 95

Glu Phe Gly Ser Arg Thr Arg Asn Val Val Phe Asp Ile Phe Thr Gly
100 105 110

Lys Gly Gln Asp Met Val Phe Thr Val Tyr Gly Glu His Trp Arg Lys
115 120 125

Met Arg Arg Ile Met Thr Val Pro Phe Phe Thr Asn Lys Val Val Gln
130 135 140

Gln Asn Arg Glu Gly Trp Glu Phe Glu Ala Ala Ser Val Val Glu Asp
145 150 155 160

Val Lys Lys Asn Pro Asp Ser Ala Thr Lys Gly Ile Val Leu Arg Lys
165 170 175

Arg Leu Gln Leu Met Met Tyr Asn Asn Met Phe Arg Ile Met Phe Asp
180 185 190

Arg Arg Phe Glu Ser Glu Asp Asp Pro Leu Phe Leu Arg Leu Lys Ala
195 200 205

Leu Asn Gly Glu Arg Ser Arg Leu Ala Gln Ser Phe Glu Tyr Asn Tyr
210 215 220

Gly Asp Phe Ile Pro Ile Leu Arg Pro Phe Leu Arg Gly Tyr Leu Lys
225 230 235 240

Ile Cys Gln Asp Val Lys Asp Arg Arg Ile Ala Leu Phe Lys Lys Tyr
245 250 255

Phe Val Asp Glu Arg Lys Gln Ile Ala Ser Ser Lys Pro Thr Gly Ser
260 265 270

Glu Gly Leu Lys Cys Ala Ile Asp His Ile Leu Glu Ala Glu Gln Lys
275 280 285

Gly Glu Ile Asn Glu Asp Asn Val Leu Tyr Ile Val Glu Asn Ile Asn
290 295 300

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Val Ala Ala Ile Glu Thr Thr Leu Trp Ser Ile Glu Trp Gly Ile Ala
305                 310                 315                 320

Glu Leu Val Asn His Pro Glu Ile Gln Ser Lys Leu Arg Asn Glu Leu
                325                 330                 335

Asp Thr Val Leu Gly Pro Gly Val Gln Val Thr Glu Pro Asp Leu His
            340                 345                 350

Lys Leu Pro Tyr Leu Gln Ala Val Val Lys Glu Thr Leu Arg Leu Arg
        355                 360                 365

Met Ala Ile Pro Leu Leu Val Pro His Met Asn Leu His Asp Ala Lys
    370                 375                 380

Leu Ala Gly Tyr Asp Ile Pro Ala Glu Ser Lys Ile Leu Val Asn Ala
385                 390                 395                 400

Trp Trp Leu Ala Asn Asn Pro Asn Ser Trp Lys Lys Pro Glu Glu Phe
                405                 410                 415

Arg Pro Glu Arg Phe Phe Glu Glu Glu Ser His Val Glu Ala Asn Gly
            420                 425                 430

Asn Asp Phe Arg Tyr Val Pro Phe Gly Val Gly Arg Arg Ser Cys Pro
        435                 440                 445

Gly Ile Ile Leu Ala Leu Pro Ile Leu Gly Ile Thr Ile Gly Arg Met
    450                 455                 460

Val Gln Asn Phe Glu Leu Leu Pro Pro Pro Gly Gln Ser Lys Val Asp
465                 470                 475                 480

Thr Ser Glu Lys Gly Gly Gln Phe Ser Leu His Ile Leu Asn His Ser
                485                 490                 495

Ile Ile Val Met Lys Pro Arg Asn Cys
            500                 505


<210> SEQ ID NO 5
<211> LENGTH: 1686
<212> TYPE: DNA
<213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 5

atggcgccac aagaacaagc agtttctcag gtgatggaga aacagagcaa caacaacaac     60

agtgacgtca ttttccgatc aaagttaccg gatatttaca tcccgaacca cctatctctc    120

cacgactaca tcttccaaaa catctccgaa ttcgccacta agccttgcct aatcaacgga    180

ccaaccggcc acgtgtacac ttactccgac gtccacgtca tctcccgcca aatcgccgcc    240

aattttcaca aactcggcgt taaccaaaac gacgtcgtca tgctcctcct cccaaactgt    300

cccgaattcg tcctctcttt cctcgccgcc tccttccgcg gcgcaaccgc caccgccgca    360

aacccttttct tcactccggc ggagatagct aaacaagcca aagcctccaa caccaaactc    420

ataatcaccg aagctcgtta cgtcgacaaa atcaaaccac ttcaaaacga cgacggagta    480

gtcatcgtct gcatcgacga caacgaatcc gtgccaatcc ctgaaggctg cctccgcttc    540

accgagttga ctcagtcgac aaccgaggca tcagaagtca tcgactcggt ggagatttca    600

ccggacgacg tggtggcact accttactcc tctggcacga cgggattacc aaaaggagtg    660

atgctgactc acaagggact agtcacgagc gttgctcagc aagtcgacgg cgagaacccg    720

aatctttatt tccacagcga tgacgtcata ctctgtgttt tgcccatgtt tcatatctac    780

gctttgaact cgatcatgtt gtgtggtctt agagttggtg cggcgattct gataatgccg    840
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aagtttgaga tcaatctgct attggagctg atccagaggt gtaaagtgac ggtggctccg    900

atggttccgc cgattgtgtt ggccattgcg aagtcttcgg agacggagaa gtatgatttg    960

agctcgataa gagtggtgaa atctggtgct gctcctcttg gtaaagaact tgaagatgcc   1020

gttaatgcca agtttcctaa tgccaaactc ggtcagggat acggaatgac ggaagcaggt   1080

ccagtgctag caatgtcgtt aggttttgca aaggaacctt ttccggttaa gtcaggagct   1140

tgtggtactg ttgtaagaaa tgctgagatg aaaatagttg atccagacac cggagattct   1200

ctttcgagga atcaacccgg tgagatttgt attcgtggtc accagatcat gaaaggttac   1260

ctcaacaatc cggcagctac agcagagacc attgataaag acggttggct tcatactgga   1320

gatattggat tgatcgatga cgatgacgag cttttcatcg ttgatcgatt gaaagaactt   1380

atcaagtata aaggttttca ggtagctccg gctgagctag aggctttgct catcggtcat   1440

cctgacatta ctgatgttgc tgttgtcgca atgaaagaag aagcagctgg tgaagttcct   1500

gttgcatttg tggtgaaatc gaaggattcg gagttatcag aagatgatgt gaagcaattc   1560

gtgtcgaaac aggttgtgtt ttacaagaga atcaacaaag tgttcttcac tgaatccatt   1620

cctaaagctc catcagggaa gatattgagg aaagatctga gggcaaaact agcaaatgga   1680

ttgtga                                                              1686
```

<210> SEQ ID NO 6
<211> LENGTH: 561
<212> TYPE: PRT
<213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 6

```
Met Ala Pro Gln Glu Gln Ala Val Ser Gln Val Met Glu Lys Gln Ser
1               5                   10                  15

Asn Asn Asn Asn Ser Asp Val Ile Phe Arg Ser Lys Leu Pro Asp Ile
            20                  25                  30

Tyr Ile Pro Asn His Leu Ser Leu His Asp Tyr Ile Phe Gln Asn Ile
        35                  40                  45

Ser Glu Phe Ala Thr Lys Pro Cys Leu Ile Asn Gly Pro Thr Gly His
    50                  55                  60

Val Tyr Thr Tyr Ser Asp Val His Val Ile Ser Arg Gln Ile Ala Ala
65                  70                  75                  80

Asn Phe His Lys Leu Gly Val Asn Gln Asn Asp Val Val Met Leu Leu
                85                  90                  95

Leu Pro Asn Cys Pro Glu Phe Val Leu Ser Phe Leu Ala Ala Ser Phe
            100                 105                 110

Arg Gly Ala Thr Ala Thr Ala Ala Asn Pro Phe Phe Thr Pro Ala Glu
        115                 120                 125

Ile Ala Lys Gln Ala Lys Ala Ser Asn Thr Lys Leu Ile Ile Thr Glu
    130                 135                 140

Ala Arg Tyr Val Asp Lys Ile Lys Pro Leu Gln Asn Asp Asp Gly Val
145                 150                 155                 160

Val Ile Val Cys Ile Asp Asp Asn Glu Ser Val Pro Ile Pro Glu Gly
                165                 170                 175

Cys Leu Arg Phe Thr Glu Leu Thr Gln Ser Thr Thr Glu Ala Ser Glu
            180                 185                 190

Val Ile Asp Ser Val Glu Ile Ser Pro Asp Asp Val Val Ala Leu Pro
        195                 200                 205
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Tyr Ser Ser Gly Thr Thr Gly Leu Pro Lys Gly Val Met Leu Thr His
    210                 215                 220

Lys Gly Leu Val Thr Ser Val Ala Gln Gln Val Asp Gly Glu Asn Pro
225                 230                 235                 240

Asn Leu Tyr Phe His Ser Asp Asp Val Ile Leu Cys Val Leu Pro Met
                245                 250                 255

Phe His Ile Tyr Ala Leu Asn Ser Ile Met Leu Cys Gly Leu Arg Val
            260                 265                 270

Gly Ala Ala Ile Leu Ile Met Pro Lys Phe Glu Ile Asn Leu Leu Leu
        275                 280                 285

Glu Leu Ile Gln Arg Cys Lys Val Thr Val Ala Pro Met Val Pro Pro
    290                 295                 300

Ile Val Leu Ala Ile Ala Lys Ser Ser Glu Thr Glu Lys Tyr Asp Leu
305                 310                 315                 320

Ser Ser Ile Arg Val Val Lys Ser Gly Ala Ala Pro Leu Gly Lys Glu
                325                 330                 335

Leu Glu Asp Ala Val Asn Ala Lys Phe Pro Asn Ala Lys Leu Gly Gln
            340                 345                 350

Gly Tyr Gly Met Thr Glu Ala Gly Pro Val Leu Ala Met Ser Leu Gly
        355                 360                 365

Phe Ala Lys Glu Pro Phe Pro Val Lys Ser Gly Ala Cys Gly Thr Val
    370                 375                 380

Val Arg Asn Ala Glu Met Lys Ile Val Asp Pro Asp Thr Gly Asp Ser
385                 390                 395                 400

Leu Ser Arg Asn Gln Pro Gly Glu Ile Cys Ile Arg Gly His Gln Ile
                405                 410                 415

Met Lys Gly Tyr Leu Asn Asn Pro Ala Ala Thr Ala Glu Thr Ile Asp
            420                 425                 430

Lys Asp Gly Trp Leu His Thr Gly Asp Ile Gly Leu Ile Asp Asp Asp
        435                 440                 445

Asp Glu Leu Phe Ile Val Asp Arg Leu Lys Glu Leu Ile Lys Tyr Lys
    450                 455                 460

Gly Phe Gln Val Ala Pro Ala Glu Leu Glu Ala Leu Leu Ile Gly His
465                 470                 475                 480

Pro Asp Ile Thr Asp Val Ala Val Val Ala Met Lys Glu Glu Ala Ala
                485                 490                 495

Gly Glu Val Pro Val Ala Phe Val Val Lys Ser Lys Asp Ser Glu Leu
            500                 505                 510

Ser Glu Asp Asp Val Lys Gln Phe Val Ser Lys Gln Val Val Phe Tyr
        515                 520                 525

Lys Arg Ile Asn Lys Val Phe Phe Thr Glu Ser Ile Pro Lys Ala Pro
    530                 535                 540

Ser Gly Lys Ile Leu Arg Lys Asp Leu Arg Ala Lys Leu Ala Asn Gly
545                 550                 555                 560

Leu
```

<210> SEQ ID NO 7
<211> LENGTH: 1176
<212> TYPE: DNA
<213> ORGANISM: Rheum tataricum

<400> SEQUENCE: 7

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```
atggcaccgg aggagtccag gcatgctgaa actgcagtta acagagccgc caccgtcctg     60

gccatcggca ctgccaaccc gccaaactgc tactatcaag cggactttcc tgacttctac    120

ttccgtgcca ccaacagcga ccacctcacg cacctcaagc aaaaatttaa gcgcatttgt    180

gagaaatcga tgattgaaaa acgttatctc catttgacgg aagaaattct caaggagaat    240

ccaaatattg cttccttcga ggcgccatca ttggatgtaa gacataacat tcaagtgaaa    300

gaagtggtgc tgctcggaaa agaggcagct ttgaaggcca tcaatgagtg gggccaaccc    360

aagtcaaaga tcacgcgcct cattgtgtgt tgtattgccg gcgttgacat gcccggcgca    420

gactatcaac tcactaaact ccttggctta caactttctg ttaagcgatt tatgttttac    480

cacctaggat gctatgccgg tggcaccgtc cttcgccttg cgaaggacat agcagaaaac    540

aacaaggaag ctcgtgttct catcgttcgc tctgagatga cgccaatctg tttccgtggg    600

ccatccgaaa cccacataga ctccatggta gggcaagcaa tatttggtga cggtgctgcg    660

gctgttatag ttggtgcaaa tcccgaccta tccatcgaaa ggccgatttt cgagttgatt    720

tctacatccc aaactatcat acctgaatcc gatggtgcga ttgagggaca tttgcttgaa    780

gttggactca gtttccaact ctaccagact gttccctcat taatctctaa ttgtatcgaa    840

acttgtcttt caaaggcttt cacacctctt aacattagtg attggaactc actattctgg    900

attgcacacc ctggtggccg tgctatcctt gacgatatcg aggctactgt tggtctcaag    960

aaggagaaac ttaaggcaac aagacaagtt ttgaacgact atgggaacat gtcaagtgct   1020

tgcgtatttt tcatcatgga tgagatgagg aagaagtcgc tcgcaaacgg tcaagtaacc   1080

actggagaag gactcaagtg gggtgttctt tttgggttcg ggccaggtgt tactgtggaa   1140

actgtggttc taagcagtgt gccgctaatt acctga                             1176
```

<210> SEQ ID NO 8
<211> LENGTH: 391
<212> TYPE: PRT
<213> ORGANISM: Rheum tataricum

<400> SEQUENCE: 8

```
Met Ala Pro Glu Glu Ser Arg His Ala Glu Thr Ala Val Asn Arg Ala
1               5                   10                  15

Ala Thr Val Leu Ala Ile Gly Thr Ala Asn Pro Pro Asn Cys Tyr Tyr
            20                  25                  30

Gln Ala Asp Phe Pro Asp Phe Tyr Phe Arg Ala Thr Asn Ser Asp His
        35                  40                  45

Leu Thr His Leu Lys Gln Lys Phe Lys Arg Ile Cys Glu Lys Ser Met
    50                  55                  60

Ile Glu Lys Arg Tyr Leu His Leu Thr Glu Glu Ile Leu Lys Glu Asn
65                  70                  75                  80

Pro Asn Ile Ala Ser Phe Glu Ala Pro Ser Leu Asp Val Arg His Asn
                85                  90                  95

Ile Gln Val Lys Glu Val Val Leu Leu Gly Lys Glu Ala Ala Leu Lys
            100                 105                 110

Ala Ile Asn Glu Trp Gly Gln Pro Lys Ser Lys Ile Thr Arg Leu Ile
        115                 120                 125

Val Cys Cys Ile Ala Gly Val Asp Met Pro Gly Ala Asp Tyr Gln Leu
    130                 135                 140
```

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Thr Lys Leu Leu Gly Leu Gln Leu Ser Val Lys Arg Phe Met Phe Tyr
145                 150                 155                 160

His Leu Gly Cys Tyr Ala Gly Gly Thr Val Leu Arg Leu Ala Lys Asp
                165                 170                 175

Ile Ala Glu Asn Asn Lys Glu Ala Arg Val Leu Ile Val Arg Ser Glu
            180                 185                 190

Met Thr Pro Ile Cys Phe Arg Gly Pro Ser Glu Thr His Ile Asp Ser
        195                 200                 205

Met Val Gly Gln Ala Ile Phe Gly Asp Gly Ala Ala Ala Val Ile Val
    210                 215                 220

Gly Ala Asn Pro Asp Leu Ser Ile Glu Arg Pro Ile Phe Glu Leu Ile
225                 230                 235                 240

Ser Thr Ser Gln Thr Ile Ile Pro Glu Ser Asp Gly Ala Ile Glu Gly
                245                 250                 255

His Leu Leu Glu Val Gly Leu Ser Phe Gln Leu Tyr Gln Thr Val Pro
            260                 265                 270

Ser Leu Ile Ser Asn Cys Ile Glu Thr Cys Leu Ser Lys Ala Phe Thr
        275                 280                 285

Pro Leu Asn Ile Ser Asp Trp Asn Ser Leu Phe Trp Ile Ala His Pro
    290                 295                 300

Gly Gly Arg Ala Ile Leu Asp Asp Ile Glu Ala Thr Val Gly Leu Lys
305                 310                 315                 320

Lys Glu Lys Leu Lys Ala Thr Arg Gln Val Leu Asn Asp Tyr Gly Asn
                325                 330                 335

Met Ser Ser Ala Cys Val Phe Phe Ile Met Asp Glu Met Arg Lys Lys
            340                 345                 350

Ser Leu Ala Asn Gly Gln Val Thr Thr Gly Glu Gly Leu Lys Trp Gly
        355                 360                 365

Val Leu Phe Gly Phe Gly Pro Gly Val Thr Val Glu Thr Val Val Leu
    370                 375                 380

Ser Ser Val Pro Leu Ile Thr
385                 390
```

<210> SEQ ID NO 9
<211> LENGTH: 1176
<212> TYPE: DNA
<213> ORGANISM: Rheum tataricum

<400> SEQUENCE: 9

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atggccccag aagagagcag gcacgcagaa acggccgtta acagagctgc aactgttttg     60

gctattggta cggccaatcc acccaattgt tactatcaag ctgactttcc tgatttttat    120

ttcagagcca caaatagcga tcatttgact catcttaagc aaaaatttaa aaggatatgc    180

gagaagtcca tgattgaaaa gagatacttg caccttaccg aagagatctt aaaagaaaac    240

ccaaatatag cttcttttga agctccctcc ttagatgtac gtcacaacat tcaagtcaag    300

gaggtggttt tacttggtaa ggaagccgca ttgaaagcta taaacgaatg gggacagcct    360

aaaagtaaga taaccagatt gatcgtatgt tgcatagctg gcgttgacat gcctggtgca    420

gattatcaac taacaaaatt gctgggtcta caattatccg taaaaaggtt tatgttctac    480

catttaggct gttacgctgg tggcacagtt ttaagactgg ctaaggatat agcagaaaat    540

aacaaggagg ctagagtctt aatagtgcgt agtgaaatga ctcctatttg ctttagaggt    600
```

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```
ccatcagaaa cacatatcga cagcatggta ggtcaggcaa ttttcggtga tggtgctgca    660

gccgtaattg tgggagctaa tcctgattta agtatcgaaa gacctatttt tgaacttatt    720

tctacttcgc aaaccattat ccccgaatca gatggtgcaa ttgaaggcca tttattggag    780

gttggtttgt cctttcaatt gtatcagaca gtgccatctt taatttcaaa ctgtatagaa    840

acctgtctaa gtaaagcatt tacaccatta aacatttctg actggaattc tttgttctgg    900

attgctcatc caggtggaag agccatctta gatgacatcg aagctactgt gggactgaaa    960

aaggaaaaac taaaagctac tagacaagtt ttaaatgact acggtaatat gtcatctgct   1020

tgtgtatttt tcattatgga tgagatgaga aaaaagtcac ttgcaaatgg ccaggtcacg   1080

acaggtgagg gtctaaaatg gggagtccta ttcggattcg gcccaggtgt cactgttgaa   1140

accgttgtcc tgtcttcggt tccattgatc acttaa                             1176
```

<210> SEQ ID NO 10
<211> LENGTH: 391
<212> TYPE: PRT
<213> ORGANISM: Rheum tataricum

<400> SEQUENCE: 10

```
Met Ala Pro Glu Glu Ser Arg His Ala Glu Thr Ala Val Asn Arg Ala
1               5                   10                  15

Ala Thr Val Leu Ala Ile Gly Thr Ala Asn Pro Pro Asn Cys Tyr Tyr
            20                  25                  30

Gln Ala Asp Phe Pro Asp Phe Tyr Phe Arg Ala Thr Asn Ser Asp His
        35                  40                  45

Leu Thr His Leu Lys Gln Lys Phe Lys Arg Ile Cys Glu Lys Ser Met
    50                  55                  60

Ile Glu Lys Arg Tyr Leu His Leu Thr Glu Glu Ile Leu Lys Glu Asn
65                  70                  75                  80

Pro Asn Ile Ala Ser Phe Glu Ala Pro Ser Leu Asp Val Arg His Asn
                85                  90                  95

Ile Gln Val Lys Glu Val Val Leu Leu Gly Lys Glu Ala Ala Leu Lys
            100                 105                 110

Ala Ile Asn Glu Trp Gly Gln Pro Lys Ser Lys Ile Thr Arg Leu Ile
        115                 120                 125

Val Cys Cys Ile Ala Gly Val Asp Met Pro Gly Ala Asp Tyr Gln Leu
    130                 135                 140

Thr Lys Leu Leu Gly Leu Gln Leu Ser Val Lys Arg Phe Met Phe Tyr
145                 150                 155                 160

His Leu Gly Cys Tyr Ala Gly Gly Thr Val Leu Arg Leu Ala Lys Asp
                165                 170                 175

Ile Ala Glu Asn Asn Lys Glu Ala Arg Val Leu Ile Val Arg Ser Glu
            180                 185                 190

Met Thr Pro Ile Cys Phe Arg Gly Pro Ser Glu Thr His Ile Asp Ser
        195                 200                 205

Met Val Gly Gln Ala Ile Phe Gly Asp Gly Ala Ala Ala Val Ile Val
    210                 215                 220

Gly Ala Asn Pro Asp Leu Ser Ile Glu Arg Pro Ile Phe Glu Leu Ile
225                 230                 235                 240

Ser Thr Ser Gln Thr Ile Ile Pro Glu Ser Asp Gly Ala Ile Glu Gly
                245                 250                 255
```

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```
His Leu Leu Glu Val Gly Leu Ser Phe Gln Leu Tyr Gln Thr Val Pro
            260                 265                 270

Ser Leu Ile Ser Asn Cys Ile Glu Thr Cys Leu Ser Lys Ala Phe Thr
        275                 280                 285

Pro Leu Asn Ile Ser Asp Trp Asn Ser Leu Phe Trp Ile Ala His Pro
    290                 295                 300

Gly Gly Arg Ala Ile Leu Asp Asp Ile Glu Ala Thr Val Gly Leu Lys
305                 310                 315                 320

Lys Glu Lys Leu Lys Ala Thr Arg Gln Val Leu Asn Asp Tyr Gly Asn
                325                 330                 335

Met Ser Ser Ala Cys Val Phe Phe Ile Met Asp Glu Met Arg Lys Lys
            340                 345                 350

Ser Leu Ala Asn Gly Gln Val Thr Thr Gly Glu Gly Leu Lys Trp Gly
        355                 360                 365

Val Leu Phe Gly Phe Gly Pro Gly Val Thr Val Glu Thr Val Val Leu
    370                 375                 380

Ser Ser Val Pro Leu Ile Thr
385                 390
```

<210> SEQ ID NO 11
<211> LENGTH: 1596
<212> TYPE: DNA
<213> ORGANISM: Rhodobacter capsulatus

<400> SEQUENCE: 11

```
atgaccctgc agtcacagac ggccaaggac tgcctcgcgc tggacggggc gctgacactt     60

gtccaatgcg aggccatcgc gacacatcgc agccggattt cggtgacccc cgcgctgcgc    120

gagcgctgcg cgcgggccca tgcccggctt gagcacgcca tcgccgagca gcgccacatt    180

tacggcatca ccaccggctt cggcccgctg gcgaaccgtc tgatcggggc cgatcagggg    240

gcggagctgc agcagaacct gatctatcat ctggccaccg gcgtcgggcc gaaactgagc    300

tgggccgagg cgcgggcgtt gatgctggcg cggctcaact cgatcctgca aggcgcgtcg    360

ggggcctcgc cggagacgat cgaccggatc gttgcggtgc tcaatgcggg gtttgccccc    420

gaggttccgg cgcagggaac ggtgggcgcc tcgggcgatc tgaccccgct tgcgcatatg    480

gtgctggcgc tgcagggacg ggggcggatg atcgacccct cgggccgcgt gcaggaggcc    540

ggggcggtga tggatcggct ctgcggcggt ccgctgacgc tggcggcccg tgacgggctg    600

gcgctggtga atggcacctc ggcgatgacc gcgattgcgg ccctgaccgg ggtcgaggcg    660

gcgcgggcga tcgacgccgc gcttcggcac agcgcggtcc tgatggaggt cttgtccggt    720

catgccgaag cctggcatcc ggctttcgca gagctgcgcc cgcatccggg gcagctgcgg    780

gcgaccgagc ggctggcgca ggcgctggat ggggcggggc gggtctgtcg gaccctgacc    840

gcggcgcggc ggctgaccgc cgcggatctg cggcccgaag atcatccggc gcaggatgcc    900

tacagtctgc gcgtggtgcc gcaactggtc ggcgcggtct gggacacgct ggactggcac    960

gatcgtgtcg tcacctgcga gctcaattcc gtcaccgaca atccgatctt tcccgagggc   1020

tgcgcggtgc ccgccctgca cggcggcaat ttcatgggcg tgcatgtcgc ccttgcctcc   1080

gatgcgctga acgcggcgct ggtgacgctg gcgggcctgg tcgagcgtca gatcgcccgg   1140

ctgaccgacg aaaagctgaa caagggcctg cccgccttcc tgcacggggg gcaggcgggg   1200

ctgcaatcgg gcttcatggg ggcgcaggtc acggcgacgg cgcttctggc ggaaatgcgg   1260
```

-continued

```
gcgaatgcca cgccggtttc ggtgcagtcg ctgtcgacca atggcgccaa tcaggatgtg   1320

gtctcgatgg gaacgattgc cgcgcggagg gcgcgggcgc agctgctgcc cctgtcgcag   1380

atccaggcga tcctggcgct tgcccttgcc caggcgatgg atctgcttga cgaccccgag   1440

gggcaggccg gatggtcgct tacggcgcgg gatctgcggg accggatccg ggcggtctcg   1500

cccgggcttc gcgccgacag accgcttgcc gggcatatcg aagcggtggc acagggtctg   1560

cgtcatccct ccgccgccgc cgatcccccg gcatga                              1596


<210> SEQ ID NO 12
<211> LENGTH: 531
<212> TYPE: PRT
<213> ORGANISM: Rhodobacter capsulatus

<400> SEQUENCE: 12

Met Thr Leu Gln Ser Gln Thr Ala Lys Asp Cys Leu Ala Leu Asp Gly
1               5                   10                  15

Ala Leu Thr Leu Val Gln Cys Glu Ala Ile Ala Thr His Arg Ser Arg
            20                  25                  30

Ile Ser Val Thr Pro Ala Leu Arg Glu Arg Cys Ala Arg Ala His Ala
        35                  40                  45

Arg Leu Glu His Ala Ile Ala Glu Gln Arg His Ile Tyr Gly Ile Thr
    50                  55                  60

Thr Gly Phe Gly Pro Leu Ala Asn Arg Leu Ile Gly Ala Asp Gln Gly
65                  70                  75                  80

Ala Glu Leu Gln Gln Asn Leu Ile Tyr His Leu Ala Thr Gly Val Gly
                85                  90                  95

Pro Lys Leu Ser Trp Ala Glu Ala Arg Ala Leu Met Leu Ala Arg Leu
            100                 105                 110

Asn Ser Ile Leu Gln Gly Ala Ser Gly Ala Ser Pro Glu Thr Ile Asp
        115                 120                 125

Arg Ile Val Ala Val Leu Asn Ala Gly Phe Ala Pro Glu Val Pro Ala
    130                 135                 140

Gln Gly Thr Val Gly Ala Ser Gly Asp Leu Thr Pro Leu Ala His Met
145                 150                 155                 160

Val Leu Ala Leu Gln Gly Arg Gly Arg Met Ile Asp Pro Ser Gly Arg
                165                 170                 175

Val Gln Glu Ala Gly Ala Val Met Asp Arg Leu Cys Gly Gly Pro Leu
            180                 185                 190

Thr Leu Ala Ala Arg Asp Gly Leu Ala Leu Val Asn Gly Thr Ser Ala
        195                 200                 205

Met Thr Ala Ile Ala Ala Leu Thr Gly Val Glu Ala Ala Arg Ala Ile
    210                 215                 220

Asp Ala Ala Leu Arg His Ser Ala Val Leu Met Glu Val Leu Ser Gly
225                 230                 235                 240

His Ala Glu Ala Trp His Pro Ala Phe Ala Glu Leu Arg Pro His Pro
                245                 250                 255

Gly Gln Leu Arg Ala Thr Glu Arg Leu Ala Gln Ala Leu Asp Gly Ala
            260                 265                 270

Gly Arg Val Cys Arg Thr Leu Thr Ala Ala Arg Arg Leu Thr Ala Ala
        275                 280                 285

Asp Leu Arg Pro Glu Asp His Pro Ala Gln Asp Ala Tyr Ser Leu Arg
```

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```
    290                 295                 300

Val Val Pro Gln Leu Val Gly Ala Val Trp Asp Thr Leu Asp Trp His
305                 310                 315                 320

Asp Arg Val Val Thr Cys Glu Leu Asn Ser Val Thr Asp Asn Pro Ile
                325                 330                 335

Phe Pro Glu Gly Cys Ala Val Pro Ala Leu His Gly Gly Asn Phe Met
            340                 345                 350

Gly Val His Val Ala Leu Ala Ser Asp Ala Leu Asn Ala Ala Leu Val
        355                 360                 365

Thr Leu Ala Gly Leu Val Glu Arg Gln Ile Ala Arg Leu Thr Asp Glu
    370                 375                 380

Lys Leu Asn Lys Gly Leu Pro Ala Phe Leu His Gly Gly Gln Ala Gly
385                 390                 395                 400

Leu Gln Ser Gly Phe Met Gly Ala Gln Val Thr Ala Thr Ala Leu Leu
                405                 410                 415

Ala Glu Met Arg Ala Asn Ala Thr Pro Val Ser Val Gln Ser Leu Ser
            420                 425                 430

Thr Asn Gly Ala Asn Gln Asp Val Val Ser Met Gly Thr Ile Ala Ala
        435                 440                 445

Arg Arg Ala Arg Ala Gln Leu Leu Pro Leu Ser Gln Ile Gln Ala Ile
    450                 455                 460

Leu Ala Leu Ala Leu Ala Gln Ala Met Asp Leu Leu Asp Asp Pro Glu
465                 470                 475                 480

Gly Gln Ala Gly Trp Ser Leu Thr Ala Arg Asp Leu Arg Asp Arg Ile
                485                 490                 495

Arg Ala Val Ser Pro Gly Leu Arg Ala Asp Arg Pro Leu Ala Gly His
            500                 505                 510

Ile Glu Ala Val Ala Gln Gly Leu Arg His Pro Ser Ala Ala Ala Asp
        515                 520                 525

Pro Pro Ala
    530


<210> SEQ ID NO 13
<211> LENGTH: 1596
<212> TYPE: DNA
<213> ORGANISM: Rhodobacter capsulatus

<400> SEQUENCE: 13

atgaccctgc aatctcaaac agctaaagat tgtttggctt tggatggtgc cttgacatta     60

gttcaatgcg aagcgatagc aacccataga agtagaatct ctgtaacacc agccctacgt    120

gagagatgtg ctagagcaca tgctaggtta gaacatgcaa tagccgaaca gcgacacata    180

tatgggataa cgacaggctt cgggccactt gctaacaggc tgatcggagc agaccagggt    240

gctgaattac aacagaacct tatctaccat ttggcaaccg gagttggccc caaattatca    300

tgggccgaag ccagagcttt aatgctcgct cgtttgaata gtatactaca aggtgcttct    360

ggtgctagcc ctgaaacaat tgataggatc gttgcagtct taaatgccgg atttgccccg    420

gaagtcccag cccaaggaac cgttggtgct tcgggtgact taactccgtt agcacacatg    480

gtattagcat tgcaaggcag aggtcgtatg attgatcctt cagggagagt tcaagaagcc    540

ggcgctgtca tggataggtt gtgtggaggc cctttaacat tggctgccag agatggcctc    600

gccttagtaa atggtacatc tgccatgaca gctattgccg cattgaccgg tgtggaggct    660
```

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gcaagagcga ttgatgcagc gcttagacat tccgcagtct tgatggaggt cctgtcaggg 720

catgctgagg cttggcaccc tgcctttgcg gaattgcgtc cgcatccagg acaattacgc 780

gccactgaga ggttagctca agcattggac ggcgcaggta gagtctgccg gactcttaca 840

gccgctaggc gtctaactgc agctgatctg agaccagaag atcatccagc tcaagatgca 900

tattcacttc gagtagttcc tcagctggtt ggtgccgtat gggatacgtt ggattggcac 960

gacagggttg tgacttgcga acttaactcc gtgaccgaca atccaatttt ccccgagggt 1020

tgtgcggttc cagcactaca cggtggaaac tttatgggcg tacatgtggc actagcttct 1080

gacgctttaa atgcagcgtt ggttacatta gctggtctag ttgaaaggca gattgcaaga 1140

cttactgatg agaagttgaa taagggtttg cctgcttttt tgcatggagg ccaagcaggt 1200

ttacaatcag gtttcatggg agctcaggtt actgctactg ctttgctagc ggaaatgaga 1260

gctaacgcga ctcccgtgtc cgttcaaagc ctcagcacca atggtgcaaa tcaagacgtg 1320

gtaagtatgg gtacgattgc cgcgagacga gcaagagctc aacttttacc tctgtctcaa 1380

atccaagcga ttttggcact ggctcttgca caagccatgg atctcctaga cgatcctgaa 1440

ggacaagccg gttggtcctt aacggcaaga gatttaagag accgtatacg ggctgtcagt 1500

ccagggttgc gcgcagatag accactagcg ggtcatattg aagctgtggc tcaaggtcta 1560

agacacccct cggcagctgc cgatccacct gcttaa 1596

<210> SEQ ID NO 14
<211> LENGTH: 531
<212> TYPE: PRT
<213> ORGANISM: Rhodobacter capsulatus

<400> SEQUENCE: 14

Met Thr Leu Gln Ser Gln Thr Ala Lys Asp Cys Leu Ala Leu Asp Gly
1 5 10 15

Ala Leu Thr Leu Val Gln Cys Glu Ala Ile Ala Thr His Arg Ser Arg
20 25 30

Ile Ser Val Thr Pro Ala Leu Arg Glu Arg Cys Ala Arg Ala His Ala
35 40 45

Arg Leu Glu His Ala Ile Ala Glu Gln Arg His Ile Tyr Gly Ile Thr
50 55 60

Thr Gly Phe Gly Pro Leu Ala Asn Arg Leu Ile Gly Ala Asp Gln Gly
65 70 75 80

Ala Glu Leu Gln Gln Asn Leu Ile Tyr His Leu Ala Thr Gly Val Gly
85 90 95

Pro Lys Leu Ser Trp Ala Glu Ala Arg Ala Leu Met Leu Ala Arg Leu
100 105 110

Asn Ser Ile Leu Gln Gly Ala Ser Gly Ala Ser Pro Glu Thr Ile Asp
115 120 125

Arg Ile Val Ala Val Leu Asn Ala Gly Phe Ala Pro Glu Val Pro Ala
130 135 140

Gln Gly Thr Val Gly Ala Ser Gly Asp Leu Thr Pro Leu Ala His Met
145 150 155 160

Val Leu Ala Leu Gln Gly Arg Gly Arg Met Ile Asp Pro Ser Gly Arg
165 170 175

Val Gln Glu Ala Gly Ala Val Met Asp Arg Leu Cys Gly Gly Pro Leu
180 185 190

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```
Thr Leu Ala Ala Arg Asp Gly Leu Ala Leu Val Asn Gly Thr Ser Ala
        195                 200                 205

Met Thr Ala Ile Ala Ala Leu Thr Gly Val Glu Ala Ala Arg Ala Ile
    210                 215                 220

Asp Ala Ala Leu Arg His Ser Ala Val Leu Met Glu Val Leu Ser Gly
225                 230                 235                 240

His Ala Glu Ala Trp His Pro Ala Phe Ala Glu Leu Arg Pro His Pro
                245                 250                 255

Gly Gln Leu Arg Ala Thr Glu Arg Leu Ala Gln Ala Leu Asp Gly Ala
            260                 265                 270

Gly Arg Val Cys Arg Thr Leu Thr Ala Ala Arg Arg Leu Thr Ala Ala
        275                 280                 285

Asp Leu Arg Pro Glu Asp His Pro Ala Gln Asp Ala Tyr Ser Leu Arg
    290                 295                 300

Val Val Pro Gln Leu Val Gly Ala Val Trp Asp Thr Leu Asp Trp His
305                 310                 315                 320

Asp Arg Val Val Thr Cys Glu Leu Asn Ser Val Thr Asp Asn Pro Ile
                325                 330                 335

Phe Pro Glu Gly Cys Ala Val Pro Ala Leu His Gly Gly Asn Phe Met
            340                 345                 350

Gly Val His Val Ala Leu Ala Ser Asp Ala Leu Asn Ala Ala Leu Val
        355                 360                 365

Thr Leu Ala Gly Leu Val Glu Arg Gln Ile Ala Arg Leu Thr Asp Glu
    370                 375                 380

Lys Leu Asn Lys Gly Leu Pro Ala Phe Leu His Gly Gly Gln Ala Gly
385                 390                 395                 400

Leu Gln Ser Gly Phe Met Gly Ala Gln Val Thr Ala Thr Ala Leu Leu
                405                 410                 415

Ala Glu Met Arg Ala Asn Ala Thr Pro Val Ser Val Gln Ser Leu Ser
            420                 425                 430

Thr Asn Gly Ala Asn Gln Asp Val Val Ser Met Gly Thr Ile Ala Ala
        435                 440                 445

Arg Arg Ala Arg Ala Gln Leu Leu Pro Leu Ser Gln Ile Gln Ala Ile
    450                 455                 460

Leu Ala Leu Ala Leu Ala Gln Ala Met Asp Leu Leu Asp Asp Pro Glu
465                 470                 475                 480

Gly Gln Ala Gly Trp Ser Leu Thr Ala Arg Asp Leu Arg Asp Arg Ile
                485                 490                 495

Arg Ala Val Ser Pro Gly Leu Arg Ala Asp Arg Pro Leu Ala Gly His
            500                 505                 510

Ile Glu Ala Val Ala Gln Gly Leu Arg His Pro Ser Ala Ala Ala Asp
        515                 520                 525

Pro Pro Ala
    530
```

<210> SEQ ID NO 15
<211> LENGTH: 2076
<212> TYPE: DNA
<213> ORGANISM: Saccharomyces cerevisiae

<400> SEQUENCE: 15

atgccgtttg gaatagacaa caccgacttc actgtcctgg cggggctagt gcttgccgtg 60

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ctactgtacg taaagagaaa ctccatcaag gaactgctga tgtccgatga cggagatatc 120

acagctgtca gctcgggcaa cagagacatt gctcaggtgg tgaccgaaaa caacaagaac 180

tacttggtgt tgtatgcgtc gcagactggg actgccgagg attacgccaa aaagttttcc 240

aaggagctgg tggccaagtt caacctaaac gtgatgtgcg cagatgttga gaactacgac 300

tttgagtcgc taaacgatgt gcccgtcata gtctcgattt ttatctctac atatggtgaa 360

ggagacttcc ccgacggggc ggtcaacttt gaagacttta tttgtaatgc ggaagcgggt 420

gcactatcga acctgaggta taatatgttt ggtctgggaa attctactta tgaattcttt 480

aatggtgccg ccaagaaggc cgagaagcat ctctccgccg cgggcgctat cagactaggc 540

aagctcggtg aagctgatga tggtgcagga actacagacg aagattacat ggcctggaag 600

gactccatcc tggaggtttt gaaagacgaa ctgcatttgg acgaacagga agccaagttc 660

acctctcaat tccagtacac tgtgttgaac gaaatcactg actccatgtc gcttggtgaa 720

ccctctgctc actatttgcc ctcgcatcag ttgaaccgca acgcagacgg catccaattg 780

ggtcccttcg atttgtctca accgtatatt gcacccatcg tgaaatctcg cgaactgttc 840

tcttccaatg accgtaattg catccactct gaatttgact tgtccggctc taacatcaag 900

tactccactg gtgaccatct tgctgtttgg ccttccaacc cattggaaaa ggtcgaacag 960

ttcttatcca tattcaacct ggaccctgaa accatttttg acttgaagcc cctggatccc 1020

accgtcaaag tgcccttccc aacgccaact actattggcg ctgctattaa acactatttg 1080

gaaattacag gacctgtctc cagacaattg ttttcatctt tgattcagtt cgcccccaac 1140

gctgacgtca aggaaaaatt gactctgctt tcgaaagaca aggaccaatt cgccgtcgag 1200

ataacctcca aatatttcaa catcgcagat gctctgaaat atttgtctga tggcgccaaa 1260

tgggacaccg tacccatgca attcttggtc gaatcagttc cccaaatgac tcctcgttac 1320

tactctatct cttcctcttc tctgtctgaa aagcaaaccg tccatgtcac ctccattgtg 1380

gaaaactttc ctaacccaga attgcctgat gctcctccag ttgttggtgt tacgactaac 1440

ttgttaagaa acattcaatt ggctcaaaac aatgttaaca ttgccgaaac taacctacct 1500

gttcactacg atttaaatgg cccacgtaaa cttttcgcca attacaaatt gcccgtccac 1560

gttcgtcgtt ctaacttcag attgccttcc aacccttcca ccccagttat catgatcggt 1620

ccaggtaccg gtgttgcccc attccgtggg tttatcagag agcgtgtcgc gttcctcgaa 1680

tcacaaaaga agggcggtaa caacgtttcg ctaggtaagc atatactgtt ttatggatcc 1740

cgtaacactg atgatttctt gtaccaggac gaatggccag aatacgccaa aaaattggat 1800

ggttcgttcg aaatggtcgt ggcccattcc aggttgccaa acaccaaaaa agtttatgtt 1860

caagataaat taaaggatta cgaagaccaa gtatttgaaa tgattaacaa cggtgcattt 1920

atctacgtct gtggtgatgc aaagggtatg gccaagggtg tgtcaaccgc attggttggc 1980

atcttatccc gtggtaaatc cattaccact gatgaagcaa cagagctaat caagatgctc 2040

aagacttcag gtagatacca agaagatgtc tggtaa 2076

<210> SEQ ID NO 16
<211> LENGTH: 691
<212> TYPE: PRT
<213> ORGANISM: Saccharomyces cerevisiae

<400> SEQUENCE: 16

-continued

```
Met Pro Phe Gly Ile Asp Asn Thr Asp Phe Thr Val Leu Ala Gly Leu
1               5                  10                  15

Val Leu Ala Val Leu Leu Tyr Val Lys Arg Asn Ser Ile Lys Glu Leu
            20                  25                  30

Leu Met Ser Asp Asp Gly Asp Ile Thr Ala Val Ser Ser Gly Asn Arg
        35                  40                  45

Asp Ile Ala Gln Val Val Thr Glu Asn Asn Lys Asn Tyr Leu Val Leu
    50                  55                  60

Tyr Ala Ser Gln Thr Gly Thr Ala Glu Asp Tyr Ala Lys Lys Phe Ser
65                  70                  75                  80

Lys Glu Leu Val Ala Lys Phe Asn Leu Asn Val Met Cys Ala Asp Val
                85                  90                  95

Glu Asn Tyr Asp Phe Glu Ser Leu Asn Asp Val Pro Val Ile Val Ser
            100                 105                 110

Ile Phe Ile Ser Thr Tyr Gly Glu Gly Asp Phe Pro Asp Gly Ala Val
        115                 120                 125

Asn Phe Glu Asp Phe Ile Cys Asn Ala Glu Ala Gly Ala Leu Ser Asn
    130                 135                 140

Leu Arg Tyr Asn Met Phe Gly Leu Gly Asn Ser Thr Tyr Glu Phe Phe
145                 150                 155                 160

Asn Gly Ala Ala Lys Lys Ala Glu Lys His Leu Ser Ala Ala Gly Ala
                165                 170                 175

Ile Arg Leu Gly Lys Leu Gly Glu Ala Asp Asp Gly Ala Gly Thr Thr
            180                 185                 190

Asp Glu Asp Tyr Met Ala Trp Lys Asp Ser Ile Leu Glu Val Leu Lys
        195                 200                 205

Asp Glu Leu His Leu Asp Glu Gln Glu Ala Lys Phe Thr Ser Gln Phe
    210                 215                 220

Gln Tyr Thr Val Leu Asn Glu Ile Thr Asp Ser Met Ser Leu Gly Glu
225                 230                 235                 240

Pro Ser Ala His Tyr Leu Pro Ser His Gln Leu Asn Arg Asn Ala Asp
                245                 250                 255

Gly Ile Gln Leu Gly Pro Phe Asp Leu Ser Gln Pro Tyr Ile Ala Pro
            260                 265                 270

Ile Val Lys Ser Arg Glu Leu Phe Ser Ser Asn Asp Arg Asn Cys Ile
        275                 280                 285

His Ser Glu Phe Asp Leu Ser Gly Ser Asn Ile Lys Tyr Ser Thr Gly
    290                 295                 300

Asp His Leu Ala Val Trp Pro Ser Asn Pro Leu Glu Lys Val Glu Gln
305                 310                 315                 320

Phe Leu Ser Ile Phe Asn Leu Asp Pro Glu Thr Ile Phe Asp Leu Lys
                325                 330                 335

Pro Leu Asp Pro Thr Val Lys Val Pro Phe Pro Thr Pro Thr Thr Ile
            340                 345                 350

Gly Ala Ala Ile Lys His Tyr Leu Glu Ile Thr Gly Pro Val Ser Arg
        355                 360                 365

Gln Leu Phe Ser Ser Leu Ile Gln Phe Ala Pro Asn Ala Asp Val Lys
    370                 375                 380

Glu Lys Leu Thr Leu Leu Ser Lys Asp Lys Asp Gln Phe Ala Val Glu
385                 390                 395                 400
```

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```
Ile Thr Ser Lys Tyr Phe Asn Ile Ala Asp Ala Leu Lys Tyr Leu Ser
                405                 410                 415

Asp Gly Ala Lys Trp Asp Thr Val Pro Met Gln Phe Leu Val Glu Ser
            420                 425                 430

Val Pro Gln Met Thr Pro Arg Tyr Tyr Ser Ile Ser Ser Ser Ser Leu
        435                 440                 445

Ser Glu Lys Gln Thr Val His Val Thr Ser Ile Val Glu Asn Phe Pro
    450                 455                 460

Asn Pro Glu Leu Pro Asp Ala Pro Pro Val Val Gly Val Thr Thr Asn
465                 470                 475                 480

Leu Leu Arg Asn Ile Gln Leu Ala Gln Asn Asn Val Asn Ile Ala Glu
                485                 490                 495

Thr Asn Leu Pro Val His Tyr Asp Leu Asn Gly Pro Arg Lys Leu Phe
            500                 505                 510

Ala Asn Tyr Lys Leu Pro Val His Val Arg Arg Ser Asn Phe Arg Leu
        515                 520                 525

Pro Ser Asn Pro Ser Thr Pro Val Ile Met Ile Gly Pro Gly Thr Gly
    530                 535                 540

Val Ala Pro Phe Arg Gly Phe Ile Arg Glu Arg Val Ala Phe Leu Glu
545                 550                 555                 560

Ser Gln Lys Lys Gly Gly Asn Asn Val Ser Leu Gly Lys His Ile Leu
                565                 570                 575

Phe Tyr Gly Ser Arg Asn Thr Asp Asp Phe Leu Tyr Gln Asp Glu Trp
            580                 585                 590

Pro Glu Tyr Ala Lys Lys Leu Asp Gly Ser Phe Glu Met Val Val Ala
        595                 600                 605

His Ser Arg Leu Pro Asn Thr Lys Lys Val Tyr Val Gln Asp Lys Leu
    610                 615                 620

Lys Asp Tyr Glu Asp Gln Val Phe Glu Met Ile Asn Asn Gly Ala Phe
625                 630                 635                 640

Ile Tyr Val Cys Gly Asp Ala Lys Gly Met Ala Lys Gly Val Ser Thr
                645                 650                 655

Ala Leu Val Gly Ile Leu Ser Arg Gly Lys Ser Ile Thr Thr Asp Glu
            660                 665                 670

Ala Thr Glu Leu Ile Lys Met Leu Lys Thr Ser Gly Arg Tyr Gln Glu
        675                 680                 685

Asp Val Trp
    690


<210> SEQ ID NO 17
<211> LENGTH: 2136
<212> TYPE: DNA
<213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 17

atgtcctctt cttcttcttc gtcaacctcc atgatcgatc tcatggcagc aatcatcaaa     60

ggagagcctg taattgtctc cgacccagct aatgcctccg cttacgagtc cgtagctgct    120

gaattatcct ctatgcttat agagaatcgt caattcgcca tgattgttac cacttccatt    180

gctgttctta ttggttgcat cgttatgctc gtttggagga gatccggttc tgggaattca    240

aaacgtgtcg agcctcttaa gcctttggtt attaagcctc gtgaggaaga gattgatgat    300

gggcgtaaga aagttaccat ctttttcggt acacaaactg gtactgctga aggttttgca    360
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aaggctttag gagaagaagc taaagcaaga tatgaaaaga ccagattcaa aatcgttgat 420

ttggatgatt acgcggctga tgatgatgag tatgaggaga aattgaagaa agaggatgtg 480

gctttcttct tcttagccac atatggagat ggtgagccta ccgacaatgc agcgagattc 540

tacaaatggt tcaccgaggg gaatgacaga ggagaatggc ttaagaactt gaagtatgga 600

gtgtttggat taggaaacag acaatatgag cattttaata aggttgccaa agttgtagat 660

gacattcttg tcgaacaagg tgcacagcgt cttgtacaag ttggtcttgg agatgatgac 720

cagtgtattg aagatgactt taccgcttgg cgagaagcat tgtggcccga gcttgataca 780

atactgaggg aagaagggga tacagctgtt gccacaccat acactgcagc tgtgttagaa 840

tacagagttt ctattcacga ctctgaagat gccaaattca atgatataaa catggcaaat 900

gggaatggtt acactgtgtt tgatgctcaa catccttaca aagcaaatgt cgctgttaaa 960

agggagcttc atactcccga gtctgatcgt tcttgtatcc atttggaatt tgacattgct 1020

ggaagtggac ttacgtatga aactggagat catgttggtg tactttgtga taacttaagt 1080

gaaactgtag atgaagctct tagattgctg gatatgtcac ctgatactta tttctcactt 1140

cacgctgaaa aagaagacgg cacaccaatc agcagctcac tgcctcctcc cttcccacct 1200

tgcaacttga gaacagcgct tacacgatat gcatgtcttt tgagttctcc aaagaagtct 1260

gctttagttg cgttggctgc tcatgcatct gatcctaccg aagcagaacg attaaaacac 1320

cttgcttcac ctgctggaaa ggatgaatat tcaaagtggg tagtagagag tcaaagaagt 1380

ctacttgagg tgatggccga gtttccttca gccaagccac cacttggtgt cttcttcgct 1440

ggagttgctc caaggttgca gcctaggttc tattcgatat catcatcgcc caagattgct 1500

gaaactagaa ttcacgtcac atgtgcactg gtttatgaga aaatgccaac tggcaggatt 1560

cataagggag tgtgttccac ttggatgaag aatgctgtgc cttacgagaa gagtgaaaac 1620

tgttcctcgg cgccgatatt tgttaggcaa tccaacttca agcttccttc tgattctaag 1680

gtaccgatca tcatgatcgg tccagggact ggattagctc cattcagagg attccttcag 1740

gaaagactag cgttggtaga atctggtgtt gaacttgggc catcagtttt gttctttgga 1800

tgcagaaacc gtagaatgga tttcatctac gaggaagagc tccagcgatt tgttgagagt 1860

ggtgctctcg cagagctaag tgtcgccttc tctcgtgaag gacccaccaa agaatacgta 1920

cagcacaaga tgatggacaa ggcttctgat atctggaata tgatctctca aggagcttat 1980

ttatatgttt gtggtgacgc caaaggcatg gcaagagatg ttcacagatc tctccacaca 2040

atagctcaag aacaggggtc aatggattca actaaagcag agggcttcgt gaagaatctg 2100

caaacgagtg gaagatatct tagagatgta tggtaa 2136

<210> SEQ ID NO 18
<211> LENGTH: 711
<212> TYPE: PRT
<213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 18

Met Ser Ser Ser Ser Ser Ser Ser Thr Ser Met Ile Asp Leu Met Ala
1 5 10 15

Ala Ile Ile Lys Gly Glu Pro Val Ile Val Ser Asp Pro Ala Asn Ala
20 25 30

Ser Ala Tyr Glu Ser Val Ala Ala Glu Leu Ser Ser Met Leu Ile Glu

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        35                  40                  45

Asn Arg Gln Phe Ala Met Ile Val Thr Thr Ser Ile Ala Val Leu Ile
    50                  55                  60

Gly Cys Ile Val Met Leu Val Trp Arg Arg Ser Gly Ser Gly Asn Ser
65                  70                  75                  80

Lys Arg Val Glu Pro Leu Lys Pro Leu Val Ile Lys Pro Arg Glu Glu
                85                  90                  95

Glu Ile Asp Asp Gly Arg Lys Lys Val Thr Ile Phe Phe Gly Thr Gln
            100                 105                 110

Thr Gly Thr Ala Glu Gly Phe Ala Lys Ala Leu Gly Glu Glu Ala Lys
        115                 120                 125

Ala Arg Tyr Glu Lys Thr Arg Phe Lys Ile Val Asp Leu Asp Asp Tyr
    130                 135                 140

Ala Ala Asp Asp Asp Glu Tyr Glu Glu Lys Leu Lys Lys Glu Asp Val
145                 150                 155                 160

Ala Phe Phe Phe Leu Ala Thr Tyr Gly Asp Gly Glu Pro Thr Asp Asn
                165                 170                 175

Ala Ala Arg Phe Tyr Lys Trp Phe Thr Glu Gly Asn Asp Arg Gly Glu
            180                 185                 190

Trp Leu Lys Asn Leu Lys Tyr Gly Val Phe Gly Leu Gly Asn Arg Gln
        195                 200                 205

Tyr Glu His Phe Asn Lys Val Ala Lys Val Val Asp Asp Ile Leu Val
    210                 215                 220

Glu Gln Gly Ala Gln Arg Leu Val Gln Val Gly Leu Gly Asp Asp Asp
225                 230                 235                 240

Gln Cys Ile Glu Asp Asp Phe Thr Ala Trp Arg Glu Ala Leu Trp Pro
                245                 250                 255

Glu Leu Asp Thr Ile Leu Arg Glu Glu Gly Asp Thr Ala Val Ala Thr
            260                 265                 270

Pro Tyr Thr Ala Ala Val Leu Glu Tyr Arg Val Ser Ile His Asp Ser
        275                 280                 285

Glu Asp Ala Lys Phe Asn Asp Ile Asn Met Ala Asn Gly Asn Gly Tyr
    290                 295                 300

Thr Val Phe Asp Ala Gln His Pro Tyr Lys Ala Asn Val Ala Val Lys
305                 310                 315                 320

Arg Glu Leu His Thr Pro Glu Ser Asp Arg Ser Cys Ile His Leu Glu
                325                 330                 335

Phe Asp Ile Ala Gly Ser Gly Leu Thr Tyr Glu Thr Gly Asp His Val
            340                 345                 350

Gly Val Leu Cys Asp Asn Leu Ser Glu Thr Val Asp Glu Ala Leu Arg
        355                 360                 365

Leu Leu Asp Met Ser Pro Asp Thr Tyr Phe Ser Leu His Ala Glu Lys
    370                 375                 380

Glu Asp Gly Thr Pro Ile Ser Ser Ser Leu Pro Pro Pro Phe Pro Pro
385                 390                 395                 400

Cys Asn Leu Arg Thr Ala Leu Thr Arg Tyr Ala Cys Leu Leu Ser Ser
                405                 410                 415

Pro Lys Lys Ser Ala Leu Val Ala Leu Ala Ala His Ala Ser Asp Pro
            420                 425                 430

Thr Glu Ala Glu Arg Leu Lys His Leu Ala Ser Pro Ala Gly Lys Asp
        435                 440                 445
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Glu Tyr Ser Lys Trp Val Val Glu Ser Gln Arg Ser Leu Leu Glu Val
    450                 455                 460

Met Ala Glu Phe Pro Ser Ala Lys Pro Pro Leu Gly Val Phe Phe Ala
465                 470                 475                 480

Gly Val Ala Pro Arg Leu Gln Pro Arg Phe Tyr Ser Ile Ser Ser Ser
                485                 490                 495

Pro Lys Ile Ala Glu Thr Arg Ile His Val Thr Cys Ala Leu Val Tyr
            500                 505                 510

Glu Lys Met Pro Thr Gly Arg Ile His Lys Gly Val Cys Ser Thr Trp
        515                 520                 525

Met Lys Asn Ala Val Pro Tyr Glu Lys Ser Glu Asn Cys Ser Ser Ala
    530                 535                 540

Pro Ile Phe Val Arg Gln Ser Asn Phe Lys Leu Pro Ser Asp Ser Lys
545                 550                 555                 560

Val Pro Ile Ile Met Ile Gly Pro Gly Thr Gly Leu Ala Pro Phe Arg
                565                 570                 575

Gly Phe Leu Gln Glu Arg Leu Ala Leu Val Glu Ser Gly Val Glu Leu
            580                 585                 590

Gly Pro Ser Val Leu Phe Phe Gly Cys Arg Asn Arg Arg Met Asp Phe
        595                 600                 605

Ile Tyr Glu Glu Glu Leu Gln Arg Phe Val Glu Ser Gly Ala Leu Ala
    610                 615                 620

Glu Leu Ser Val Ala Phe Ser Arg Glu Gly Pro Thr Lys Glu Tyr Val
625                 630                 635                 640

Gln His Lys Met Met Asp Lys Ala Ser Asp Ile Trp Asn Met Ile Ser
                645                 650                 655

Gln Gly Ala Tyr Leu Tyr Val Cys Gly Asp Ala Lys Gly Met Ala Arg
            660                 665                 670

Asp Val His Arg Ser Leu His Thr Ile Ala Gln Glu Gln Gly Ser Met
        675                 680                 685

Asp Ser Thr Lys Ala Glu Gly Phe Val Lys Asn Leu Gln Thr Ser Gly
    690                 695                 700

Arg Tyr Leu Arg Asp Val Trp
705                 710


<210> SEQ ID NO 19
<211> LENGTH: 33
<212> TYPE: DNA
<213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 19

cggaattctc atggatcaaa tcgaagcaat gtt                                33


<210> SEQ ID NO 20
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 20

cgactagttt agcaaatcgg aatcggagc                                     29


<210> SEQ ID NO 21
<211> LENGTH: 30
<212> TYPE: DNA
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<213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 21

cgctcgagat atggacctcc tcttgctgga 30

<210> SEQ ID NO 22
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 22

cgggtacctt aacagttcct tggtttcata ac 32

<210> SEQ ID NO 23
<211> LENGTH: 36
<212> TYPE: DNA
<213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 23

gctctagacc tatggcgcca caagaacaag cagttt 36

<210> SEQ ID NO 24
<211> LENGTH: 36
<212> TYPE: DNA
<213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 24

gcggatcccc ttcacaatcc atttgctagt tttgcc 36

<210> SEQ ID NO 25
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Rheum tataricum

<400> SEQUENCE: 25

ccggatccaa atggccccag aagagagcag g 31

<210> SEQ ID NO 26
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Rheum tataricum

<400> SEQUENCE: 26

cgctcgagtt aagtgatcaa tggaaccgaa gacag 35

<210> SEQ ID NO 27
<211> LENGTH: 38
<212> TYPE: DNA
<213> ORGANISM: Rhodobacter capsulatus

<400> SEQUENCE: 27

ccgaattccc atgaccctgc aatctcaaac agctaaag 38

<210> SEQ ID NO 28
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Rhodobacter capsulatus

<400> SEQUENCE: 28

ccactagttt aagcaggtgg atcggcagct 30

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<210> SEQ ID NO 29
<211> LENGTH: 37
<212> TYPE: DNA
<213> ORGANISM: Saccharomyces cerevisiae

<400> SEQUENCE: 29

ccctcgagat catgccgttt ggaatagaca acaccga 37

<210> SEQ ID NO 30
<211> LENGTH: 36
<212> TYPE: DNA
<213> ORGANISM: Saccharomyces cerevisiae

<400> SEQUENCE: 30

ccaagcttat cgggctgatt accagacatc ttcttg 36

<210> SEQ ID NO 31
<211> LENGTH: 36
<212> TYPE: DNA
<213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 31

ccggatcccc atgtcctctt cttcttcttc gtcaac 36

<210> SEQ ID NO 32
<211> LENGTH: 34
<212> TYPE: DNA
<213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 32

ccctcgaggt gagtgtgtgg cttcaatagt ttcg 34

<210> SEQ ID NO 33
<211> LENGTH: 40
<212> TYPE: DNA
<213> ORGANISM: Rhodobacter capsulatus

<400> SEQUENCE: 33

ccgctcgagc ggatgaccct gcaatctcaa acagctaaag 40

<210> SEQ ID NO 34
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Rhodobacter capsulatus

<400> SEQUENCE: 34

gcggatcctt aagcaggtgg atcggcagct 30

<210> SEQ ID NO 35
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 35

tgccatggca atggcgccac aagaacaagc agttt 35

<210> SEQ ID NO 36
<211> LENGTH: 36
<212> TYPE: DNA
<213> ORGANISM: Arabidopsis thaliana

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<400> SEQUENCE: 36

gcggatcccc ttcacaatcc atttgctagt tttgcc 36

<210> SEQ ID NO 37
<211> LENGTH: 37
<212> TYPE: DNA
<213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 37

ttgcggccgc aaatctcgat cccgcgaaat taatacg 37

<210> SEQ ID NO 38
<211> LENGTH: 36
<212> TYPE: DNA
<213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 38

cgctcgagcc ttcacaatcc atttgctagt tttgcc 36

<210> SEQ ID NO 39
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: S. cerevisiae

<400> SEQUENCE: 39

gtattctata tccacgcctg caaac 25

<210> SEQ ID NO 40
<211> LENGTH: 45
<212> TYPE: DNA
<213> ORGANISM: S. cerevisiae

<400> SEQUENCE: 40

agtacataca gggaacgtcc ctacaggaac gcaaacttaa gctac 45

<210> SEQ ID NO 41
<211> LENGTH: 50
<212> TYPE: DNA
<213> ORGANISM: S. cerevisiae

<400> SEQUENCE: 41

gcatacaatc aactatctca tatacaatgc cgtttggaat agacaacacc 50

<210> SEQ ID NO 42
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: S. cerevisiae

<400> SEQUENCE: 42

gcttccgcat tacaaataaa gtcttcaa 28

<210> SEQ ID NO 43
<211> LENGTH: 42
<212> TYPE: DNA
<213> ORGANISM: S. cerevisiae

<400> SEQUENCE: 43

ggacgttccc tgtatgtact agggggatcg aagaaatgat gg 42

<210> SEQ ID NO 44

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<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: S. cerevisiae

<400> SEQUENCE: 44

gagcaatgaa cccaataacg aaatc 25

<210> SEQ ID NO 45
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: S. cerevisiae

<400> SEQUENCE: 45

cttgacgttc gttcgactga tgagc 25

<210> SEQ ID NO 46
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: S. cerevisiae

<400> SEQUENCE: 46

tgtatatgag atagttgatt gtatgc 26

<210> SEQ ID NO 47
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: K. lactis

<400> SEQUENCE: 47

cttgacgttc gttcgactga tgagc 25

<210> SEQ ID NO 48
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: K. lactis

<400> SEQUENCE: 48

ctggaattcg atgatgtagt ttctgg 26

<210> SEQ ID NO 49
<211> LENGTH: 42
<212> TYPE: DNA
<213> ORGANISM: S. cerevisiae

<400> SEQUENCE: 49

ctacatcatc gaattccagc tacgtatggt catttcttct tc 42

<210> SEQ ID NO 50
<211> LENGTH: 49
<212> TYPE: DNA
<213> ORGANISM: S. cerevisiae

<400> SEQUENCE: 50

tttttgatta aaattaaaaa aacttttag tttatgtatg tgtttttg 49

<210> SEQ ID NO 51
<211> LENGTH: 50
<212> TYPE: DNA
<213> ORGANISM: S. cerevisiae

<400> SEQUENCE: 51

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agtttttta attttaatca aaaaatgagc gaagaaagct tattcgagtc 50

<210> SEQ ID NO 52
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: S. cerevisiae

<400> SEQUENCE: 52

cacctaaaga cctcatggcg ttacc 25

<210> SEQ ID NO 53
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: K. lactis

<400> SEQUENCE: 53

cggtctgcat tggatggtgg taac 24

<210> SEQ ID NO 54
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: K. lactis

<400> SEQUENCE: 54

gagcaatgaa cccaataacg aaatc 25

<210> SEQ ID NO 55
<211> LENGTH: 42
<212> TYPE: DNA
<213> ORGANISM: S. cerevisiae

<400> SEQUENCE: 55

ctacatcatc gaattccagc tacgtatggt catttcttct tc 42

<210> SEQ ID NO 56
<211> LENGTH: 44
<212> TYPE: DNA
<213> ORGANISM: S. cerevisiae

<400> SEQUENCE: 56

caccatccaa tgcagaccgt tttagtttat gtatgtgttt tttg 44

<210> SEQ ID NO 57
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: S. cerevisiae

<400> SEQUENCE: 57

tgttctgctc tcttcaattt tcctttc 27

<210> SEQ ID NO 58
<211> LENGTH: 43
<212> TYPE: DNA
<213> ORGANISM: S. cerevisiae

<400> SEQUENCE: 58

ctggaattcg atgatgtagt ttctaatttt ctgcgctgtt tcg 43

<210> SEQ ID NO 59
<211> LENGTH: 1083
<212> TYPE: DNA

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<213> ORGANISM: S. cerevisiae

<400> SEQUENCE: 59

atgcttaaca gaacaattgc taagagaact ttagccactg ccgctcaggc ggaacgcacc 60

ctacccaaga agtatggcgg tcgtttcacc gtcactttga tacctggtga cggtgttggg 120

aaagaaatca ctgattcagt gagaaccatt tttgaggctg aaaatatccc gatcgactgg 180

gaaactataa acattaagca aacagatcat aaggaaggcg tctatgaagc tgttgagtct 240

ctaaagagaa ataagattgg tcttaagggg ctatggcaca ctcctgctga ccaaacaggt 300

cacggttcac taaacgttgc tttgcgtaaa caactagata tctacgccaa tgtggccctt 360

ttcaaatcct tgaagggtgt caagactaga attccagaca tagatttgat tgtcattaga 420

gaaaacacgg agggtgagtt ctcaggcctg gaacatgaat ccgtccctgg tgtagtggaa 480

tctttgaaag ttatgactag acctaagaca gaaaggatcg ccagatttgc ctttgacttc 540

gccaagaaat acaacagaaa gtctgtcaca gctgtgcata aggcaaatat catgaagtta 600

ggtgacggtc tgttcagaaa tataataact gaaattggcc aaaaagaata tcctgatatt 660

gacgtatcgt ccatcattgt cgacaatgcc tccatgcagg cggtggccaa acctcatcaa 720

tttgatgtcc tagttacccc ttcaatgtac ggtaccatct taggcaacat tggcgctgct 780

ttgatcggtg gtccaggatt ggtggcaggt gccaactttg gcagggacta tgctgtcttc 840

gaaccaggtt ccagacatgt tggtttagat attaaaggcc aaaatgtggc taacccaact 900

gccatgatcc tttcctccac gttaatgttg aaccatttgg gtttgaatga atatgctact 960

agaatctcaa aggcagttca tgaaacgatc gcagaaggta agcataccac tagagatatt 1020

ggtggttcct cttctactac tgacttcacg aatgaaatca tcaacaaatt atctaccatg 1080

taa 1083

<210> SEQ ID NO 60
<211> LENGTH: 1671
<212> TYPE: DNA
<213> ORGANISM: A. thaliana

<400> SEQUENCE: 60

atgacgacac aagatgtgat agtcaatgat cagaatgatc agaaacagtg tagtaatgac 60

gtcattttcc gatcgagatt gcctgatata tacatcccta accacctccc actccacgac 120

tacatcttcg aaaatatctc agagttcgcc gctaagccat gcttgatcaa cggtcccacc 180

ggcgaagtat acacctacgc cgatgtccac gtaacatctc ggaaactcgc cgccggtctt 240

cataacctcg gcgtgaagca acacgacgtt gtaatgatcc tcctcccgaa ctctcctgaa 300

gtagtcctca ctttccttgc cgcctccttc atcggcgcaa tcaccacctc cgcgaacccg 360

ttcttcactc cggcggagat ttctaaacaa gccaaagcct ccgcggcgaa actcatcgtc 420

actcaatccc gttacgtcga taaaatcaag aacctccaaa acgacggcgt tttgatcgtc 480

accaccgact ccgacgccat ccccgaaaac tgcctccgtt tctccgagtt aactcagtcc 540

gaagaaccac gagtggactc aataccggag aagatttcgc cagaagacgt cgtggcgctt 600

cctttctcat ccggcacgac gggtctcccc aaaggagtga tgctaacaca caaaggtcta 660

gtcacgagcg tggcgcagca agtcgacggc gagaatccga atctttactt caacagagac 720

gacgtgatcc tctgtgtctt gcctatgttc catatatacg ctctcaactc catcatgctc 780

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tgtagtctca gagttggtgc cacgatcttg ataatgccta agttcgaaat cactctcttg 840

ttagagcaga tacaaaggtg taaagtcacg gtggctatgg tcgtgccacc gatcgtttta 900

gctatcgcga agtcgccgga gacggagaag tatgatctga gctcggttag gatggttaag 960

tctggagcag ctcctcttgg taaggagctt gaagatgcta ttagtgctaa gtttcctaac 1020

gccaagcttg gtcagggcta tgggatgaca gaagcaggtc cggtgctagc aatgtcgtta 1080

gggtttgcta aagagccgtt tccagtgaag tcaggagcat gtggtacggt ggtgaggaac 1140

gccgagatga agatacttga tccagacaca ggagattctt tgcctaggaa caaacccggc 1200

gaaatatgca tccgtggcaa ccaaatcatg aaaggctatc tcaatgaccc cttggccacg 1260

gcatcgacga tcgataaaga tggttggctt cacactggag acgtcggatt tatcgatgat 1320

gacgacgagc ttttcattgt ggatagattg aaagaactca tcaagtacaa aggatttcaa 1380

gtggctccag ctgagctaga gtctctcctc ataggtcatc cagaaatcaa tgatgttgct 1440

gtcgtcgcca tgaaggaaga agatgctggt gaggttcctg ttgcgtttgt ggtgagatcg 1500

aaagattcaa atatatccga agatgaaatc aagcaattcg tgtcaaaaca ggttgtgttt 1560

tataagagaa tcaacaaagt gttcttcact gactctattc ctaaagctcc atcagggaag 1620

atattgagga aggatctaag agcaagacta gcaaatggat taatgaacta g 1671

<210> SEQ ID NO 61
<211> LENGTH: 40
<212> TYPE: DNA
<213> ORGANISM: A. thaliana

<400> SEQUENCE: 61

gcgaattctt atgacgacac aagatgtgat agtcaatgat 40

<210> SEQ ID NO 62
<211> LENGTH: 42
<212> TYPE: DNA
<213> ORGANISM: A. thaliana

<400> SEQUENCE: 62

gcactagtat cctagttcat taatccattt gctagtcttg ct 42

<210> SEQ ID NO 63
<211> LENGTH: 392
<212> TYPE: PRT
<213> ORGANISM: Vitis vinifera

<400> SEQUENCE: 63

Met Ala Ser Val Glu Glu Phe Arg Asn Ala Gln Arg Ala Lys Gly Pro
1 5 10 15

Ala Thr Ile Leu Ala Ile Gly Thr Ala Thr Pro Asp His Cys Val Tyr
20 25 30

Gln Ser Asp Tyr Ala Asp Tyr Tyr Phe Arg Val Thr Lys Ser Glu His
35 40 45

Met Thr Glu Leu Lys Lys Lys Phe Asn Arg Ile Cys Asp Lys Ser Met
50 55 60

Ile Lys Lys Arg Tyr Ile His Leu Thr Glu Glu Met Leu Glu Glu His
65 70 75 80

Pro Asn Ile Gly Ala Tyr Met Ala Pro Ser Leu Asn Ile Arg Gln Glu
85 90 95

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Ile Ile Thr Ala Glu Val Pro Arg Leu Gly Arg Asp Ala Ala Leu Lys
            100                 105                 110

Ala Leu Lys Glu Trp Gly Gln Pro Lys Ser Lys Ile Thr His Leu Val
        115                 120                 125

Phe Cys Thr Thr Ser Gly Val Glu Met Pro Gly Ala Asp Tyr Lys Leu
    130                 135                 140

Ala Asn Leu Leu Gly Leu Glu Thr Ser Val Arg Arg Val Met Leu Tyr
145                 150                 155                 160

His Gln Gly Cys Tyr Ala Gly Gly Thr Val Leu Arg Thr Ala Lys Asp
                165                 170                 175

Leu Ala Glu Asn Asn Ala Gly Ala Arg Val Leu Val Val Cys Ser Glu
            180                 185                 190

Ile Thr Val Val Thr Phe Arg Gly Pro Ser Glu Asp Ala Leu Asp Ser
        195                 200                 205

Leu Val Gly Gln Ala Leu Phe Gly Asp Gly Ser Ser Ala Val Ile Val
    210                 215                 220

Gly Ser Asp Pro Asp Val Ser Ile Glu Arg Pro Leu Phe Gln Leu Val
225                 230                 235                 240

Ser Ala Ala Gln Thr Phe Ile Pro Asn Ser Ala Gly Ala Ile Ala Gly
                245                 250                 255

Asn Leu Arg Glu Val Gly Leu Thr Phe His Leu Trp Pro Asn Val Pro
            260                 265                 270

Thr Leu Ile Ser Glu Asn Ile Glu Lys Cys Leu Thr Gln Ala Phe Asp
        275                 280                 285

Pro Leu Gly Ile Ser Asp Trp Asn Ser Leu Phe Trp Ile Ala His Pro
    290                 295                 300

Gly Gly Pro Ala Ile Leu Asp Ala Val Glu Ala Lys Leu Asn Leu Glu
305                 310                 315                 320

Lys Lys Lys Leu Glu Ala Thr Arg His Val Leu Ser Glu Tyr Gly Asn
                325                 330                 335

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<212> TYPE: DNA
<213> ORGANISM: Vitis vinifera

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ccaaatatag gtgcatatat ggcaccatct ttgaatatta gacaagaaat cataacagcc    300

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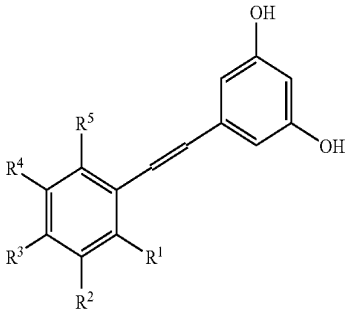
1. A method for the production of a cis- or trans-stilbenoid of the general formula 1:

Formula 1



in which each of R^1R^2, R^3, R^4 and R^5 independently is hydrogen or hydroxy, or a glycosylated or oligomeric form thereof, comprising cultivating a micro-organism producing said stilbenoid, wherein said cultivation is performed in a multi-phase system comprising at least an aqueous first phase containing said micro-organism and a second phase immiscible with said aqueous phase in which said stilbenoid accumulates.

2. A method as claimed in claim 1, wherein said stilbenoid constitutes said second phase.

3. A method as claimed in claim 1, wherein said first phase is continuous and said second phase is dispersed therein.

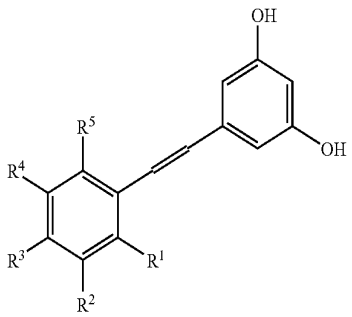
4. A method as claimed in claim 1, wherein said second phase is a liquid.

5. A method as claimed in claim 4, wherein said second phase is a micro-encapsulated liquid.

6. A method as claimed in claim 1, wherein said micro-organism, when cultivated in said aqueous phase without said immiscible phase, is capable of producing said stilbenoid in an amount sufficient to reach a saturated concentration thereof in said aqueous phase and to precipitate therefrom.

7. A method for the production of a cis- or trans-stilbenoid of the general formula 1:

Formula 1



in which each of R^1, R^2, R^3, R^4 and R^5 independently is hydrogen or hydroxy, or a glycosylated or oligomeric form thereof, comprising cultivating a micro-organism producing said stilbenoid, wherein said cultivation is conducted in a culture medium comprising or consisting of an aqueous phase and produces an amount of said stilbenoid released from the micro-organisms into the culture medium which exceeds the solubility limit of said stilbenoid in said aqueous phase.

8. A method as claimed in claim 7, wherein said stilbenoid precipitates from said culture medium.

9. A method as claimed in claim 7, wherein said cultivation is performed in a said culture medium which is a multi-phase system comprising at least said aqueous phase containing said micro-organism and a liquid solvent immiscible with said aqueous phase in which said stilbenoid accumulates.

10. A method as claimed in claim **9**, wherein said liquid solvent forms a liquid-liquid interface with said aqueous phase.

11. A method as claimed in claim **1**, wherein said stilbenoid is resveratrol (only R^3=OH), pinosylvin (all R groups are hydrogen) or piceatannol (only R^3 and either R^2 or R^4 is OH).

12. A method as claimed in claim **1**, wherein said stilbenoid is trans.

13. A method as claimed in claim **1**, wherein said liquid or said solvent comprises an ester.

14. A method as claimed in claim **13**, wherein said ester is of the general formula R^6—COO—R^7, and R^6 is H or an aliphatic straight or branched chain hydrocarbon moiety of from 1-6 carbon atoms and R^7 is an aliphatic straight or branched chain hydrocarbon moiety of from 2-16 carbon atoms, or a heteroatom containing hydrocarbon moiety of from 2 to 16 carbon atoms or an aromatic or heteroaromatic moiety of from 5 to 16 carbon atoms.

15. A method as claimed in claim **14**, wherein R^7 has from 3 to 9 carbon atoms.

16. A method as claimed in claim **14**, wherein R^6 has from 1 to 4 carbon atoms.

17. A method as claimed in claim **14**, wherein said ester is an octyl acetate.

18. A method as claimed in claim **1**, wherein said liquid comprises an alkane.

19. A method as claimed in claim **18**, wherein said alkane is a C_6 to C_{16} straight or branched chain alkane.

20. A method as claimed in claim **19**, wherein said alkane is n-dodecane.

21. A method as claimed in claim **1**, further comprising separating said second phase and extracting said stilbenoid therefrom.

22. A method as claimed in claim **1**, further comprising an additional step of recovering said stilbenoid.

23. A method for producing an extraction solvent tolerant micro-organism strain producing a metabolite comprising:

(a) cultivating a starting micro-organism in a multi-phase system comprising at least an aqueous first phase containing said micro-organism and a second phase immiscible with said aqueous phase in which said metabolite accumulates, said second phase comprising a first solvent component to which the micro-organism is more tolerant and a first concentration of a second solvent component to which the micro-organism is less tolerant,

(b) recovering progeny micro-organism from said cultivation (a), and

(c) culturing said progeny micro-organism in a said multi-phase system in which the concentration of said second solvent component is increased above said first concentration.

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